

Does Britain belong to Europe?

Margaret Thatcher's speech last week was not nearly as bad as her most fierce critics say, but not nearly as good as it should have been.

Mrs Thatcher's speech on the future of Europe, last week at Bruges, earned her a bad press in the sense that the only praise for it was from British chauvinists, but that is no doubt what she intended. Her reference to herself as a kind of Genghis Khan on the issue of European integration shows that she may even relish the hostility — and the attention — her speech has evoked. That she may have misjudged the present mood of her own electorate, now more inclined to European collaboration than at any time since Churchill offered constitutional union with France in 1940 (and was rebuffed) is neither here nor there. It would, however, have been more prudent of Mrs Thatcher to have allowed that the constituency of those who argue for a United States of Europe is not as self evidently utopian as she claimed last week. Part of the difficulty lies in her style; believing as she evidently does that the presentation of an argument in public, even to an academic audience, need consist merely of a statement of her own conclusions in very simple language, she appears at once dogmatic and simplistic.

But Mrs Thatcher's critics have also jumped too quickly to the conclusion that her speech at Bruges was an implacable assertion that national sovereignty must always take precedence over supranational considerations. Mrs Thatcher is at worst a gradualist, if a grudging one. She would have won more friends last week if she had tempered her protests at interference in national affairs by the European Commission in Brussels with a more sympathetic account of what she would positively welcome.

Transition

On one point, for example, Mrs Thatcher is indisputably correct: Europe is in transition, but nobody is sure what the end point will be. What has so far been agreed is that the ten members of the European Community should function as an unfettered free-trade area — the substance of the Treaty of Rome signed 33 years ago. The present excitement centres on the ambition that trade should become genuinely free by 1992, several decades later than intended. Why the delay? Despite the altruism and enthusiasm of the 1950s, experience has shown that national governments have been more willing to seize the benefits of access to others' markets than to reciprocate. Some governments have openly subsidized domestic industries to give them a better chance of winning sales elsewhere in Europe. Some have kept out imports by non-fiscal means (tariffs as such are illegal), including simple bureaucracy and health or environmental legislation. The readiness of governments to favour domestic suppliers has been a scandal. Apparently prudent legislation in the fields of environment, public health and financial services has been used to prevent the internationalization of, say, the insurance industry. The plan for 1992 is in essence that the European Commission should have the power, under the supervision of the member governments, to turn this morass into what is called a level playing-field.

Mrs Thatcher was wrong, last week, in failing to acknowledge that this process entails some erosion of national sovereignty. British chauvinism was outraged, in June, that Brussels intervened to prevent the British government writing off all the debt of a nationalized motor-manufacturer (Austin-Rover) which it

planned to sell, on the grounds that the result would have been an unfair subsidy (which is true), but has been mollified to learn that much the same is likely to happen in the French government's dealings with Renault, which it owns. It would make a nonsense of the notion of free-trade Europe if the commission were not so empowered. Equally, it will be essential that there should be common regulation of the degree to which financial institutions should be backed by capital resources; otherwise, weaker institutions will be best able to capture new business, increasing the risks for those who deal with them. It was therefore simplistic of Mrs Thatcher to complain, without discrimination, that the European Commission has acquired regulatory powers. It would have been more useful if she had tried more carefully (and more sympathetically) to distinguish between necessary and unnecessary regulation from the centre.

How level?

The objective, it will be recalled, is to abolish restraints on trade. As things are, this is the only general principle on which the member states of the European Community are agreed, although the European Council of Ministers, on which all member governments have an equal say, can agree to extend this remit, issue by issue, and has the power to decide which of the commission's attempts at common regulation should go ahead. The general issue, at the heart of the dispute between Mrs Thatcher and the commission, is that of how level a level playing-field should be. In the past, the commission has tried (with some success) to regulate environmental pollution by means of emission standards, on the grounds that any other policy would give industries sited where standards are more lenient an unfair economic advantage. Now it is talking of harmonizing taxation rates and social benefits for the same reasons.

However desirable uniformity in these fields might be for other reasons, slavish harmonization may even contradict the goal of an efficient division of labour within the European Community. Countries in which the environment is more able to absorb pollution, or whose people are more willing to put up with it, are the best places in which to site polluting industries (provided there are no international repercussions). Governments levying high taxes to pay for generous social benefits will presumably drive industry elsewhere as, in the United States of America, New York found to its cost in the early 1970s. In short, a more coherent case can be made for resisting some (but not all) central regulation than anybody would have thought from listening to Mrs Thatcher's views last week.

But Mrs Thatcher was on sure ground last week in her demand that single-market Europe should not be protectionist. That is not an issue that will be decided as a whole, but will emerge from a series of compromises among the member states on particular, even tiny, questions. Sadly, even the British government is not as single-minded on the principle as its prime minister claims. The Common Agricultural Policy (which Mrs Thatcher roundly criticized last week) shelters farmers from more efficient producers elsewhere at a cost comparable with that



of subsidizing farmers in the United States (roughly \$30,000 million a year).

But keeping 'out' motor-cars and electronic goods manufactured in Japan is a common enterprise, the General Agreement on Tariffs and Trade (GATT) notwithstanding. It would be easier to believe that the European enterprise for manufacturing the Airbus range of aircraft does not infringe the GATT rules if the accounts were publicly available. When 1992 arrives, Europe will be filled with the wailing of nascent industries claiming special protection in Europe's long-term interest. The publication last week of the agreed European standard for high-definition television equipment, most notable for its incompatibility with Japanese and US standards, is a depressing sign of which way the wind is blowing. One of the unfortunate effects of Mrs Thatcher's barely qualified distrust of Brussels and all its works is that she may be less able than she would wish to exert an enlightening influence on trade protection in the three critical years that lie ahead.

Defence

On many other European issues, Britain's prime minister appears to be equally correct, even if her case is too simply put to be convincing. She is, for example, right to say that, if the objective were indeed to create the United States of Europe, it would be more sensible to start at the other end of the process the federalists now see stretching ahead, with arrangements for mutual defence. One present difficulty is that two members of the community (France and Ireland) do not belong to the North Atlantic Treaty Organisation (NATO). Last week, she stated as if self-evident the need for more spending on conventional forces and more general acceptance of nuclear deterrence in European defence. The snag is that the case, which is strong, would be more persuasive if it were put by somebody who is enthusiastic about some other field for European cohesion.

Mrs Thatcher was also right to remind her audience at Bruges that the European states, not all of which belong to the European Community, have a common and interesting history and cultural tradition. What she may have underestimated is the degree to which the mere existence of the community, and the likelihood that its plans for free trade and free movement will at last be realized, will themselves become powerful contributors to the same historical and cultural processes. To insist, as Mrs Thatcher did last week, that Europe must take one step at a time is sensible, but is not necessarily (for a politician) prudent. The rhetoric of national destiny and even the concept of sovereignty will probably seem even more old-fashioned by 1992. That is why it would have been clever to have anticipated some of the opportunities that by then will have arisen.

Four years from now, the important interest will centre on quite different issues: the European roles of political and cultural institutions, basic research and higher education in particular. Even now, it is clear that the device of the Council of Ministers is mostly a means of reaching the least offensive compromises between national interests, while the European Parliament at Strasbourg remains ineffectual. Even taken together, they are not a sufficiently democratic way of administering even the single market planned for 1992. How will they be changed? Who will even bring up that awkward question?

Higher education and research are a different case. While even Mrs Thatcher applauds Europe's cultural heritage, there are very few who appreciate how much is now being done by universities and scholars further to enrich that tradition. Yet the intellectual professions, traditionally mobile as they are, and more ready than most to acknowledge their dependence on others, have been surprisingly quick to seize on the opportunities provided by the European community. It is only necessary to see how Spanish universities have become part of the general swim in just two years to recognize that reality — and its benefits. Similarly, the number of research institutes and labora-

tories which have shaken themselves free of strictly national roots by building international reputations is growing steadily. The commission's more expensive programmes of research support, most of which seem designed to encourage inter-company collaboration that should be replaced, in 1992, by competition, would even now be better spent in supporting these cultural, but rewarding, pursuits. □

Hacking is a crime

Is unauthorized access to other people's computers a crime, and how should it be dealt with?

THE modern world is well known to be full of earnest young teenagers huddled over personal computers which they use for telling what other computer users store on their machines. In the past several years, there have been some spectacular cases of electronic burglary by these means. Understandably, hackers (as the young people concerned are called) seem most of all determined to break into the computers of the most mighty — defence and intelligence networks are prime targets, certain to yield a rich harvest of publicity if not of data. Often the hackers, being engaging as well as ingenious young people, are dealt with indulgently, perhaps even asked to inspect the hardware they have broken into. Occasionally, they find themselves in trouble with the law. But not seriously, because the law is at best ambiguous.

That is the starting-point for an intelligent discussion, now published, by the British government's Law Commission, the body of lawyers that advises on the need for legislation and on the operation of what is already on the statute book. On this occasion, the commission is not proposing that there should be specific legislation, but is merely raising for more general discussion, the prospect that there might be. Naturally enough, it begins from the premise that computers are but machinery of a kind, which is fair enough. It goes on to argue that it is not a criminal offence, at least under British law, to use a piece of machinery belonging to somebody else provided that the machine, which might be a motor car or a lawnmower, is returned to its owner undamaged. Nor, the commission says, is it a crime at present to gain unauthorized access to information (although that may change if the British government's proposals on the reform of the Official Secrets Act come into being, at least for some categories of data). Naturally, those borrowing other people's machines without permission will tend to use them for purposes or in ways that are themselves criminal, while getting hold of data without authority is often a prelude to criminal acts such as fraud. But, the argument continues, those are the acts that under present law make people liable to criminal prosecution.

So far, so good, but in any case the authors of the Law Commission's report are evidently in two minds about the issue. Although the analogy between computers and other kinds of machinery may be a useful starting-point, there is plainly a vast difference in the uses that may be made by nefarious borrowers of computers or of the information they contain. Borrowed machinery of other kinds tends to be mobile, conspicuous and closely associated with the unauthorized user; computers, on the other hand, rarely ring warning bells when a hacker has gained unauthorized entry to them, deliberate corruption of stored data is not easily identifiable, and the uses to which inside information may be put enable people to perpetrate crimes more surreptitiously than in ordinary life. That is why the Law Commission's discussion paper is likely to persuade many of its readers that the case for legislation to prohibit the deliberate entry into other people's computer systems is overwhelming. The snag, for the British government, is that such a law would veer close to privacy legislation, from which British governments have traditionally shied. □

No alternative in sight for animals in research

■ Benefits said to outweigh social cost

■ No unanimous conclusion by committee

Washington

THE National Research Council (NRC) this week has taken its turn at trying to resolve the difficult issues surrounding the use of animals in research. After three years of hearings and discussions on the question, a special committee appointed by the Commission of Life Sciences concluded, although not unanimously, that the "appropriate and humane use" of animals in research should continue.

The NRC report* cost \$300,000 to produce, and as data are not yet available from the Institute of Laboratory Animal Resources, relies on previous studies by the Department of Agriculture and the Office of Technology Assessment to reach the conclusion that between 17 and 22 million animals are used annually in research. Of these, an estimated 85 per cent are rats and mice, with cats, dogs and non-human primates at less than two per cent of the total.

Alternatives to the use of animals should be considered by scientists, according to the NRC report, and federal agencies should move rapidly to adopt such tests once available. But the report concludes that "the chance that alternatives will completely replace animals in the foreseeable future is nil".

On the question of regulations for the proper care of animals, the NRC committee concluded that existing regulations were adequate if properly enforced. But this issue proved a divisive one. Christine Stevens, president of the Animal Welfare Institute and a member of the NRC committee, refused to sign the report, and added a statement published as an appendix to the report which argues that the committee had failed to face "the widespread, ingrained problem of unnecessary suffering among millions of laboratory animals".

A second appended statement, written by Arthur Guyton of the University of Mississippi School of Medicine, sets out an opposite viewpoint, criticizing the NRC committee for failing to take a stronger stand against repressive regulations foisted on the research community by the animal rights movement.

Norman Hackerman, a physical chemist who has served as president of both the University of Texas and of Rice University, chaired the NRC committee. He says that he and others on the committee who had no need of animals in their research — and therefore no personal bias in the sub-

ject — nonetheless were persuaded that the benefits derived from animal research outweighed the social cost.

The NRC report concluded that pound animals should be made available for research purposes. But Congress may be ready to set its own agenda on this issue. The Senate has passed and sent to the House of Representatives the Pet Theft Act, which would apply restrictions to the use of 'random source' dogs and cats. The act restricts the sale to researchers of dogs and cats obtained from public or private pounds, research facilities or from individuals who have bred and raised animals on their own property. This is aimed at stopping animal auctions and other quick sales that might encourage pet thefts. The act would also require pounds to hold animals for at least seven days before selling them, a restriction that would be prohibitively expensive for pounds according to opponents of the bill.

The House agriculture subcommittee on research is scheduled to hold hearings on the bill this week. **Joseph Palca**

**Use of Laboratory Animals in Biomedical and Behavioral Research. National Academy Press, Washington, DC, 1988.*

■ ANIMAL rights groups are waging a local fight to block the sale of stray animals by Los Angeles County pounds. A law suit against the county, filed by the New York-based Fund for Animals and Los Angeles-based Actors and Others for Animals in September 1986, was argued in court last week. Los Angeles county pounds currently sell animals under a county ordinance permitting the use of strays for "humanely conducted" research. The groups asked the court to prohibit such sales, charging that the county does not adequately ensure that the research conducted on pound animals is humane.

George Baca, deputy director of the county animal control department, defended the sale of strays, claiming that safeguards in the system prevent lost pets from getting "caught up in the mill." Every animal that comes into a county pound is held for seven days in case it is claimed by its owner, and then is offered up for adoption as a pet before it is offered for research. Baca said public pressure against the sale of pound animals has decreased the demand for animals in recent years. Of 85,000 animals that came into Los Angeles county shelters in the past year, only 700 were sold for research.

Marcia Barinaga

More cash for NIH

PRESIDENT Ronald Reagan last week signed the National Institutes of Health appropriations bill, providing \$7,200 million for the NIH in the 1989 fiscal year, compared with \$6,300 million in the current fiscal year.

\$606 million of the NIH appropriation is included in total of \$1,224 million appropriated to the Public Health Service specifically for dealing with the AIDS problem. The Centers for Disease Control gets \$382 million, with the rest spread among the Alcohol, Drug Abuse and Mental Health Administration (\$175.5 million), the Health Resources and Services Administration (\$45.5 million) and the office of the Assistant Secretary for Health (\$13.4 million). **J.P.**

Acid questions

ACID rain is causing soils in Britain to become increasingly acidic, but there is no proof that it is responsible for forest decline.

That is one of the main conclusions of the first report on the UK Terrestrial Effects Review Group, established by the Department of the Environment in 1984, on the effect of acid rain on the terrestrial environment, which was published last week. Changes in soil biology may result from increasing soil acidity, and this is likely to alter plant nutrition and to change the chemistry and biology of freshwaters. The report concludes that this should be a high priority area for research in Britain.

C.McG.

Gold for Nozières

THE Centre National de la Recherche Scientifique (CNRS), France's major state research organization, has awarded its 1988 Gold Medal to Philippe Nozières. Nozières, aged 56, holds the chair of statistical physics at the Collège de France. He was awarded the CNRS Silver Medal in 1962 and was the first French physicist to receive the Wolf Prize. He is most celebrated for his contributions to the physics of metals, semiconductors and magnetism. **P.C.**

Mysterious beauty

BENOIT Mandelbrot, pioneer of fractal geometry, has been awarded the 1988 Louis Vuitton-Moët-Hennessy 'Science for Art' prize, worth FF100,000 (\$16,000). This is the first year that LVMH have offered a Science for Art prize, for important contributions to 'aesthetics research'. According to LVMH, "the mysterious beauty of fractal images is witness to the intimate relation between art and science".

Mandelbrot was born in Warsaw and educated at the Ecole Polytechnic in Paris. In 1958 he joined the IBM Research Center in the USA, where he is now an IBM fellow. **P.C.**

AIDS bill wins overwhelming Congressional support

Washington

AFTER almost two years of disagreements, debates and compromises the US House of Representatives last week finally passed its first major bill designed to help stop the spread of AIDS. Only a handful of conservative Republicans offered opposition to the boosting of AIDS research, testing and counselling.

Moves are already under way to join the bill with a separate bill, passed by the Senate in April, that gives top emphasis to AIDS education. The resulting comprehensive measure has a good chance of going to the White House for signing before Congress adjourns in early October. Although the Reagan administration has opposed the bill, the strength of Congressional support makes a presidential veto unlikely.

The bill sets a precedent in requiring the federal agencies involved in research on AIDS and the development and review of AIDS drugs to hire more staff. A total of 780 new posts are provided with the major beneficiaries certain to be the National Institutes of Health, the Centers for Disease Control and the Food and Drug Administration. Increased priority is given to AIDS research by requirements that research grants be reviewed within six months of the closing date for applications, and that requests from federal agencies for personnel and facilities be answered within 14 days.

Delays in licensing new drugs have been a major source of Congressional concern. Under a last-minute amendment, added by the bill's principal sponsor Henry Waxman (Democrat, California), experimental drugs will become more easily available to people with AIDS. Medical practitioners will be permitted to prescribe experimental drugs for AIDS sufferers, provided the manufacturer has gained an exemption for investigational use of the drug. At present, access to such drugs is controlled by clinical protocols. A further relaxation of the regulations is likely in the future with legislation under discussion that would allow terminally ill people free access to unapproved drugs once they are determined to be safe.

A series of measures aims at speeding up research. One sets up an international data bank at the National Library of Medicine. Another provides for the development of an epidemiological data base at the Centers for Disease Control. All states that receive money under the bill are required to collect demographic information about those who test positive for the AIDS virus.

The most controversial section of the bill provides \$400 million a year for testing

and pre- and post-test counselling of people who have a high risk of exposure to the HIV virus. Differences of opinion centred around the need for compulsory testing and the confidentiality of test results. Conservatives, led by William Dannemeyer (Republican, California), backed a series of amendments that would have forced testing of all 550,000 members of the US prison population, all those applying for marriage licences, and those entering hospital. But the three amendments were rejected on the grounds that

they would cost a lot but yield little useful result. Pre-marriage tests were particularly criticized because of the damage that can be done by false-positive results. But prisoners at high risk will be tested for the HIV virus under a separate amendment to the bill.

Dannemeyer also introduced an amendment that would require the names of those testing positive to be recorded and their contacts traced. But the majority view was that anonymity must be protected in order that the maximum number of people at risk come forward for testing. Statistics from Oregon were cited showing that the number of prostitutes and homosexual men seeking tests rose rapidly after state requirements that their names be reported were eliminated.

Alun Anderson

■ While conservative Republican Congressman William Dannemeyer did not win support for his views in the House of Representatives, he may do better in his home state of California. Voters in California currently appear to favour a state-wide proposition, sponsored by Dannemeyer, that would require doctors, blood banks and others to report to local health officials the names of HIV-positive individuals. The initiative, similar in style to Dannemeyer-sponsored amendments rejected by Congress, also calls for the tracing of all sexual contacts who may have contracted HIV.

Because it would require the reporting of HIV-infected patients participating in research projects, opponents say the initiative would paralyse research into potential AIDS drugs and vaccines. The cost to the state for reporting and partner-tracing was projected by two professors in the UC Berkeley School of Public Policy to exceed \$772 million.

The initiative, called Proposition 102, is slated for a vote in November. Its co-sponsor is Paul Gann, a former state legislator best known for leading the California tax-revolt, who contracted AIDS through a blood transfusion. It is the third proposition of its kind to come before California voters. In November 1986 and June 1988, voters rejected initiatives, backed by supporters of right-wing extremist Lyndon LaRouche, which called for the reporting and quarantine of HIV-infected people.

Despite the defeat of the initiative in June, a poll in late July showed 72 per cent of voters in favour of Proposition 102, when it was described to them simply as an initiative to require the reporting to local health officials of the names of people infected with HIV. Opponents of the proposition — who include all Californian medical associations — are relying on an information campaign to change voters' minds. Polls show that support for the proposition vanishes when voters are told of its potential cost and consequences.

Marcia Barinaga

Biological warfare lab dropped

Washington

YIELDING to pressure from local residents and anti-biotechnology activist Jeremy Rifkin, the US army last week announced that it will drop its plans to build a high-containment weapons laboratory at its Dugway Proving Ground in Utah. Instead the army intends to construct a less sophisticated facility that would not be suitable for testing the microbes which most worry opponents: genetically engineered microbes and those which cause incurable diseases.

The army has contended that the tests it plans to conduct at the Dugway facility do not require the highest containment level, biosafety level 4 (BL-4), but that it would prefer to build such a facility in case its needs change, and as an added degree of safety. It played down the option of building a lower-containment biosafety level 3 (BL-3) laboratory on these grounds in the environmental impact statement on its biological defence activities. That impact statement was produced as a result of a lawsuit brought by Rifkin in 1985 (see *Nature* 331, 647; 1988).

Ironically, lowering the safety designation of the laboratory is not likely to get the army off the hook. Grassroots opposition to building any sort of biological warfare test facilities at Dugway is strong, and is being coordinated at the Federal level by Rifkin. Rifkin says he will pressure the army to prepare another full environmental impact statement on the BL-3 laboratory — a process that could delay construction for two more years — and that if the army "so much as lays one brick" he will petition the courts for an injunction. But officials at Dugway say that they only intend to "expand the discussion of (a BL-3 laboratory) as the preferred alternative" in the original environmental impact statement, and that they will include costs for the laboratory in Dugway's budget request to Congress beginning in 1991. Carol Ezzell

Japan ready for home-grown commercial satellite services

Tokyo

JAPAN'S National Space Development Agency (NASDA) successfully launched a communications satellite last week, opening the way to commercial satellite services in Japan. But the satellite and a twin launched earlier this year will face strong competition from US-made satellites to be launched shortly by private corporations in Japan.

The 0.5-tonne Sakura-3b satellite was launched on Friday from NASDA's Tanegashima space centre by an H-1 rocket. If all goes according to plan, in about 3 months the satellite will be placed in geostationary orbit alongside Sakura-3a, launched in February.

Most of the satellites' communication channels will be used by Nippon Telegraph and Telephone (NTT) to establish telecommunications links with remote Japanese islands and alternative nationwide trunk lines. But 13 other government and private organizations will use the satellites for purposes ranging from emergency communications during disasters to in-house video transmissions

Japan's mini-shuttle sinks before lift-off

Tokyo

AN attempt by Japan's Institute of Space and Astronautical Sciences (ISAS) to launch a mini-shuttle into the upper atmosphere failed last week when the shuttle sank in the Pacific Ocean with its booster rocket still primed for blast-off.

The 2-m experimental shuttle lifted off from the institute's space centre in Kysuhu cradled in a gondola that dangled beneath a

between branches of private companies.

The competition will begin to show itself next year. Japan Communications Satellite Co. (JC-Sat), a joint venture of the Japanese trading giants C. Itoh and Mitsui & Co. with Hughes Communications Inc. of the United States, will launch two giant satellites next year, one with a European Ariane rocket, the other on a US Titan rocket.

Similarly, the Space Communications Corporation established by Mitsubishi Corporation and Mitsubishi Electric plans to launch two satellites next year built by Ford Aerospace of the United States.

The JC-Sat satellites will be more than twice the size of the Sakura twins, with 32 as opposed to 12 transponders each. Furthermore, the US-made transponders will cost only about two-thirds as much as those developed in Japan. And NTT, which is now privatized, has hinted that in future it may not use satellites developed by the Japanese government.

Nevertheless, Japan's Science and Technology Agency, to which NASDA is affiliated, is pushing on with development of the next generation of communications satellites. The agency has asked for over ¥6,000 million (\$45 million) to develop the Engineering Test Satellite VI, a giant 2-tonne communications satellite scheduled for launch in 1992. David Swinbanks

Shuttle launch set for today

Washington

THE space shuttle *Discovery* is due to be launched today, 29 September, marking the resumption of the US space programme more than two and a half years after the explosion of *Challenger*. Safety procedures for the launch have been tightened, and flight controllers will not waive any of the requirements.

Despite some concern last week when it was discovered that the booster which underwent the final ground test had been fitted with an O-ring of old design, *Discovery* is as mechanically well-prepared as any vehicle that the National Aeronautics and Space Administration (NASA) has launched. The five crew members, led by 47-year-old Frederick Hauck, are all veterans of previous shuttle flights.

Although the time since the *Challenger* accident has been taken up with a complete overhaul of the shuttle's design, there are still many unanswered questions about the purpose of the shuttle program. The revised safety management procedures mean that shuttle flights will be more expensive and less frequent than was originally planned, and the notion of the shuttle as an economical conveyor of cargo into space has been lost. But NASA's efforts mean that the shuttle will be the main focus of the agency for some time yet. David Lindley

Satellite launch marks Israel's calling card to the space age

Tel Aviv

ISRAEL launched its first satellite, the Ofeq 1, last week, becoming the first Middle Eastern power with the capacity to gather its own intelligence on neighbouring countries from space.

Israeli leaders across the political spectrum immediately praised the launch. Professor Yuval Ne'eman, chairman of the Israeli Space Agency, called it "our calling card to the space age". Ofeq lifted off from a beach south of Tel Aviv and is being monitored by the MBT Systems and Space Technology plant, part of Israel Aircraft Industry's (IAI) electronics division.

Officials claim that the 153-kg Ofeq is an 'experimental' satellite, and that the current purely civilian project carried only instruments for monitoring the spacecraft's own performance. Ne'eman denied reports that it was carrying a 3-kg payload for surveillance purposes.

IAI claims the purpose of the launch is to "demonstrate a capability to orbit a satellite and to check the functional ability

of its subsystems in a space environment".

Many local and foreign observers believe that this test launch paves the way for Israel to send a military satellite into space, reducing reliance on the United States for intelligence information. An added motivation for developing the necessary technology, some sources suggest, was concern that the United States was filtering some information gathered by its own satellites and withholding it from Israel. Ne'eman does not comment on these suggestions. But he does say that the current practice of conducting scientific and technical experiments via the satellites of other countries, predominantly the United States, will continue for some time. But when the next Israeli satellite is ready, it will carry two scientific experiments now being developed. Ne'eman hopes the next launch will be "within a year or two, depending on economics". He said that the success of Israel's satellites will be partly determined by IAI's ability to find customers for its launch services. Lisa Perlman

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Japanese mini-shuttle

giant balloon. The balloon was supposed to rise to a height of 20 km at which point the shuttle's booster rocket would have been fired to blast the shuttle to a height of 80 km for a test re-entry into the Earth's atmosphere. But after reaching a height of only 18 km the balloon began to descend, and ISAS scientists were forced to separate the balloon from the gondola which parachuted back to Earth. When the gondola splashed down in the Pacific the \$2 million shuttle sank. David Swinbanks

Dangers of industrial domination of academic research

London

GOVERNMENT science policies which encourage 'industrialization' of academic research and aim at national leadership in science and technology may be seriously flawed, according to this year's annual report of the Organisation for Economic Cooperation and Development (OECD) on science and technology policy. In many countries, industry is substantially expanding its research activity and governments are pressing academic institutions to form closer links with industry. The result is increasing commercialization of scientific knowledge and a system of research that is more practical, short-term and commercial in its orientation. But the report says both academics and industrialists are concerned about how far this should go. "Traditional research systems, with universities at their core, have served the broad public interest very well in many countries. Radical change which compromises the primary function of academic institutions — education and basic research — may not serve these interests."

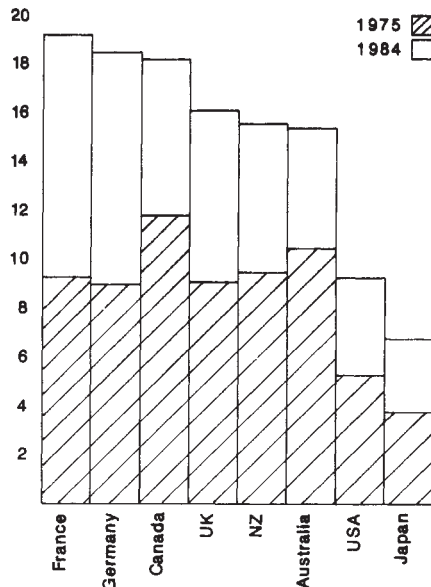
Business enterprises now account for a substantial, and the most rapidly growing, part of total national expenditures on R&D, says the report. In Japan and the United States in particular, industry has become a major supporter of basic research. In Japan, almost 35 new laboratories have been opened in the last 3–4 years.

But the report disagrees with governments which believe that by encouraging industry to support research in academic institutions, public expenditure on research could be reduced. Although industrial investment is increasing, it is still relatively small, is directed to only a narrow segment of research fields, and is focused on short-term returns on investments. And because of the 'explosive' growth of opportunities for important basic research only a fraction of which is being funded, there is little scope for reducing the overall amount of research support to universities as a result of rising industrial funding.

The OECD is also concerned that the growing 'industrialization' of research is threatening the accessibility of research results. "The prompt and open publication of basic scientific research has worked well and the practice should not be discarded lightly", it says. But the trend towards increasing confidentiality of results has already begun. Decisions concerning publication often rest with the industrial sponsors of academic research and institutions sometimes agree to delay publication for a year, to allow the company to apply for patents. Changes in intellectual property rules would ensure results were protected

as well as being accessible, says the report.

The OECD also criticizes government policies on international collaboration as being out of touch with changes taking place within both universities and industry. Academic institutions are cooperating more with foreign institutions and companies, as are industrial firms. But government policies do not reflect this trend, and are based on the belief that the national



Percentage of research publications with foreign co-authors.

interests are best served by relying on the country's own research system.

Aiming for national leadership in science and technology is unrealistic because no country can be self-sufficient and no country can isolate its research; benefits flow quickly to other countries through the organizations which perform the research. The OECD says each government should consider the policies and priorities of other countries in formulating national strategies. "The challenge for governments is to mesh the efforts with the international system so as to exploit the full potential of the latter."

The report also analyses in depth another area of weakness in the policy of most governments: the application of new technology. It says most countries place too much emphasis of innovation at the expense of diffusion of technologies into industry. Only in Japan is the balance between the two fairly even. Many other countries regard government involvement in diffusion as unnecessary interference with the market. But the OECD says that countries which cannot develop their own technologies should be concentrating their resources on diffusing technologies elsewhere.

Christine McGourty

German maglev trains way ahead of rivals

Hamburg

JAPAN may have captured the publicity — and the world speed records — for magnetically levitated trains, but West Germans believe they will win the race for successful commercial operation. Magnetically levitated trains have been running on a 31.5 km test track in Lower Saxony since 1983 and the federal government has now decided to build the world's first inter-city track for commercial traffic. It will link Hanover and Hamburg, and trains will cover the 155 kilometres in just 30–40 minutes.

The builders, a consortium including Thyssen Henschel, Messerschmidt-Bölkow-Blohm and Krauss-Maffei, want to demonstrate the feasibility of the new trains under real conditions. They claim to be five years ahead of the Japanese companies who are their only competitors.

The German "Transrapid" train uses conventional magnets, rather than the superconducting magnets favoured by the Japanese. Wing-like traps fold under a central T-shaped guideway beneath the train to pull the car a centimetre into the air by magnetic attraction. The Japanese train, in contrast, sits in a U-shaped guideway lined with aluminium coils. Magnetic repulsion pushes the car ten centimetres off the ground.

The track between Hamburg and Hanover will cost DM 1,800 million (\$1,000 million) and will be ready by the mid-1990s. But long before then, industry hopes to win orders from abroad. Under consideration are maglev routes between Moscow and Leningrad in the Soviet Union, Mecca and Medina in Saudi Arabia, and Ottawa and Montreal in Canada. A definite possibility is a link between Los Angeles and Las Vegas, which would cut the travel time from five hours by car to 70 minutes by train.

But not all West German companies are happy about the train. The Transrapid TR-07, now under construction, will reach 500 km per hour, and could so improve on air travel times within Germany as to take business away from Lufthansa.

The federal railway company Deutsche Bundesbahn is also worried by the train. The company runs at an enormous loss — its total debt is close to DM 45,000 million (\$24,000 million) — and has spent huge sums on a new rapid train called Inter-City Experimental. A superfast competitor could render these rapid trains obsolete; in hilly country maglev track costs about DM 30 million per kilometre against Inter-City Experimental's DM 40 million.

Jürgen Neffe

Harvard officials in struggle against union representation

Boston

HARVARD University officials have challenged a vote held earlier this year that would make AFSCME (American Federation of State, County and Municipal Employees) the official bargaining unit for its approximately 4,000 clerical and technical workers. The National Labor Relations Board is expected to make a decision later this year in what has become a bitter labour struggle.

Harvard officials, who have consistently opposed the union, claim that union organizers violated the letter of labour law by the way they kept track of who had voted in the union plebiscite. The administration's complaint has narrowed from an earlier claim that union organizers coerced and intimidated voters into endorsement of the union.

On 17 May, with 93 per cent of the workers casting ballots, clerical and technical staff at Harvard voted in favour of the union by a margin of 44 votes. Remarkably, those 44 votes represented the largest margin ever won by a union at a private university in the United States.

The fight for union representation has focused on issues of self-representation and participation. The union made much of the high turnover of workers at the university, which ranges between departments from 25 per cent to 40 per cent. Rallying under the slogan "we can't eat prestige", the union organizers have been stressing not only the standard union issues of economic security, but also those of job satisfaction.

Some campus scientists have echoed the

university's position against the union because they fear losing the flexibility to dismiss technicians whose work is unacceptable. Administration officials argued in a well funded campaign that a union would hurt university policy of merit-based pay in favour of promotion based on seniority and other criteria. University President Derek C. Bok has received special attention for his outspoken opposition to the union, which represents a shift from his well known former defence of pro-labour positions.

Union representatives acknowledge that salaries at Harvard tend to be higher overall than those at many other universities, and concede that the university has tended to be an 'average' employer in terms of wages and benefits. But chief organizer Kristine Rondeau says the university's recent claims of unfairness in the vote amount to stonewalling. She says that Harvard's position since the vote indicates that the university wants "to crush the union", saying that it is "willing to do almost anything to achieve that goal".

Robert Scott, university vice-president for finance, defends Harvard's complaints. "Our position is that it is not in the best interest of Harvard, or of the people who work here, to endorse the union", he says, "but it is their choice." He says that if the labour board, in its decision expected before the end of the year, finds the election to have been fair, then Harvard will willingly work with the union. But the issue may not be resolved, as neither Scott nor Rondeau rules out the possibility of an appeal against the decision.

Seth Shulman

Dark horse in superconductor patent race

Tokyo

A NEW competitor has entered the field in the complex international race to win patent rights to high-temperature superconductors. Last week, the newsletter *Nikkei Superconductors* revealed that Professor Kazuo Fueki, formerly of Tokyo University, filed a patent application for lanthanum-based superconductors on 22 December 1986, long before more famous contenders at IBM, AT&T and Houston University.

Fueki says his patent application has been filed in the United States as well as in Japan and is for lanthanum-strontium and lanthanum-calcium copper oxides. He and his colleagues at Tokyo University made them by substituting strontium and calcium for barium in the material described in Bednorz and Muller's Nobel prize-winning work. The patent applica-

tion also describes the crystal structure of the lanthanum-based superconductors.

As the patent is only for lanthanum-based compounds, it may be of little commercial value. But it seems certain to complicate the struggle for patent rights to the more valuable yttrium-based superconductors (see *Nature* 335, 4; 1988).

AT&T and Paul Chu's group at Houston University are attempting to string together patent applications for yttrium- and lanthanum-based materials and date them from early January 1987, when they filed patent applications for the lanthanum-based materials. Although these early patent applications allow for the possibility of yttrium substitution, patents were not filed specifically for yttrium-based superconductors until after the materials were discovered by Chu's group in late January 1987. David Swinbanks

First scientific fraud conviction

Washington

IN THE first criminal conviction for scientific fraud to come before a US court, a 36-year old psychologist last week pleaded guilty to falsifying data that he presented to the federal agency funding his research.

The psychologist, Stephen Breuning, was working at the University of Pittsburgh when doubts were first raised about his research on the treatment of hyperactivity in mentally retarded children. Until recently he was assistant director at the Polk Center, a Pennsylvania state institution for the mentally retarded. He now faces a possible fine of \$10,000 and five years in prison on each of two charges after pleading guilty to making false statements to a federal agency at a court in Baltimore, Maryland.

The case is particularly serious because Breuning's research had a rapid impact on the choice of drugs used to treat certain behavioural disorders in mentally retarded children. As Robert Sprague, his accuser, put it, "the issue of scientific fraud in research on psychotropic medications . . . is not an academic game, but directly influences the lives and welfare of tens of thousands of mentally retarded people".

Sprague is a professor at the University of Illinois at Urbana-Champaign and an expert in the study of hyperactive and mentally retarded children. But despite the potential seriousness of the case, he had great difficulty in getting anyone to listen to his accusations. He first suspected that something was wrong with Breuning's work when he visited his laboratory in 1983 and noted an impossibly perfect level of agreement in independently judged behavioural scores. Later in the year, he wrote to the National Institute of Mental Health (NIMH) detailing his observations and pointing out that it was impossible to do the number of experiments claimed by Breuning in the time allotted for them. The complaints were passed to the University of Pittsburgh and Breuning apparently admitted that some statements concerning earlier work were false. But no investigation was made by the university of Breuning's work at Pittsburgh.

Three years after writing his first letter Sprague became "thoroughly disgusted" with the lack of action by NIMH and contacted *Science* magazine. Shortly after an article appeared there, NIMH issued a draft report. A final report followed four months later and stated that "Breuning knowingly, wilfully and repeatedly engaged in misleading and deceptive practices". Breuning will be sentenced in early November, close to five years from the time Sprague first wrote to NIMH.

Alun Anderson

Glasnost not fully practised

London

DESPITE official Soviet encouragement of *glasnost*, general implementation of the policy remains haphazard. Although some 90 per cent of the stocks of foreign periodicals in Soviet libraries have now been transferred to open access (to the chagrin of librarians who have thus lost their bonuses for dealing with classified material), some 40 per cent of Soviet periodicals have been subjected to a quota system arising from what is described officially as a lack of newsprint.

The periodicals whose subscriptions for 1989 will be restricted include many that have played a prominent part in recent disclosures in the Soviet press. Part of the official explanation is that a number of pulp mills have been closed on environmental grounds.

A more serious problem is the habit of secrecy engrained in Soviet enterprises, in which the space programme is especially culpable according to *Pravda's* science correspondent, A. Pokrovski. While medals and awards are no longer conferred in secret, the space planners remain unnecessarily security-conscious.

Pokrovski says that Soviet hopes of earning hard currency by launching foreign satellites have been thwarted because Western insurers cannot obtain data on the reliability of Soviet launchers. Industrial spin-off from the space programme is rare, he writes, despite official assurances that space research is meant to benefit the national economy.

New pieces of space hardware described in *Pravda* are said to evoke floods of letters asking when the new pump, alloy or medical monitoring device will be generally available — but in vain. Photographs and telemetry images from space similarly fail to reach the agricultural or forestry experts who could use them, although similar materials are available — at a price — for almost any region of the world, from space agencies less obsessed with secrecy.

The Soviet Union's partners in space do not share this caution. Czechoslovakia, not normally regarded as being at the forefront of *glasnost*, has allowed the lasers developed by the Czech Technical University for the Interkosmos ranging programme to be adapted for surgical use.

Only in cartography is there a breakthrough in the use of Soviet space surveys. New maps have been promised which, for the first time since 1917, will reflect the genuine contours of Soviet cities. They will, presumably, also correct deliberate errors in the latitude and longitude of major cities, which historian Roy Medvedev has reported.

Vera Rich

French research budget increase may have long-term drawbacks

Paris

AN INCREASE of 7.6 per cent in the French civil research and development budget for 1989, announced at the beginning of August (see *Nature* 334, 556; 1988) has now been given government approval. In the short term, the budget will lift morale in public sector research, giving French science and technology a boost in the run-up to a free-market Europe in 1992. But this state investment depends on receipts from France's highly idiosyncratic tax structure, which may have to be revised if plans to implement a common European taxation system in 1993 are upheld.

President François Mitterrand promised, in his spring electoral campaign, to make research and education a national priority and so this budget comes as no surprise. While tax incentives for industrial research, initially established under the socialists but greatly extended under the Chirac government, remain untouched, Prime Minister Michel Rocard shares Mitterrand's faith in the public sector.

Between 1966 and 1988, civil research spending dropped by 3.7 per cent and recruitment was effectively halted. Much of the current increase in spending is aimed at redressing this net downward trend. This year, 597 new research posts and 316 engineer and technician positions have been created, compared to 150 research and no technician posts in 1988.

The technology development agency ANVAR receives a 15 per cent increase in grants.

But where is the money coming from to pay for these increases? This generosity with the public purse is bold, given that Mitterrand has also promised to reduce the national debt, just as the full cost of France's enthusiastic engagement in the European space programme will start to be felt. Evidently, some cuts have been made. Grants to industry as a whole have dropped by 0.62 per cent, whereas the atomic energy commission (CEA) receives 5.2 per cent less than last year and almost 6,000 defence jobs are to be lost.

Overall, Rocard's 1989 budget is more than covered by an increase in France's gross domestic product (5.1 per cent). Tax receipts, particularly from value-added tax on goods and services, are also up on last year. And it is this revenue which largely supports the current expansion in public sector spending. But French taxation is out of step with almost every other European country. Value-added tax is high (an average of 19.9 per cent compared to 15.9 per cent in Britain), whereas income tax is low (an average of 12.7 per cent). If European nations push ahead with plans to introduce a uniform tax structure in 1993, the French economy will be faced with severe problems of adjustment.

Peter Coles

More money needed to beat pollution

Washington

ADMINISTRATORS at the US Environmental Protection Agency (EPA) have unveiled plans to bolster basic research on the prevention and reduction of environmental pollutants. The plans would double the agency's research budget and shift its focus from monitoring and cleaning up pollution at the "end of the pipeline" to the development of strategies for reducing the amount of pollution created in the first place.

The plans were drawn up at the request of EPA administrator Lee Thomas by a committee composed of members of the agency's Science Advisory Board, the governmental equivalent of a corporate board of directors. Thomas asked the committee to advise on the direction the agency should take over the long-term, given that the sources of pollution are becoming increasingly decentralized and that disposal sites for hazardous materials are quickly being exhausted.

The committee concluded that regulating industrial emissions and waste is no longer sufficient, and that the EPA should

take a larger role in promoting research into ways to reduce the amount of pollutants being produced. The committee's ten recommendations centre on reorganizing the EPA administration to give more prominence to long-term research.

Specifically, the committee suggested creating an Environmental Research Institute to lead a national programme of ecological research, setting up a council to define core research areas, and changing the EPA Assistant Administrator for Research and Development from a political to a career position.

To pay for the changes, the committee recommends that EPA's \$317 million research budget be doubled over the next 5 years. While such an increase may seem extravagant, the committee says that the sum is small compared with the \$70,000 million the United States now spends on pollution control each year. But the Reagan administration is winding down, and the fate of the plans depends on who is appointed head of the EPA by the next occupant of the White House. Carol Ezzell

Evaluating nuclear accidents

SIR—Your news columns reported a “scale of severity” to evaluate nuclear accidents, as proposed by the French Industry Minister Alan Madelin¹. Madelin’s scale is intended to allow a layman to appreciate the degrees of risk involved in designing large, risky projects, but has been criticized as giving a false image of objectivity. His six-level scale is defined mainly in terms of the extent of pollution risk, which, in the case of nuclear reactors, corresponds dominantly with the amount of radioactivity released during a potential accident. But a more serious shortcoming in Madelin’s scale is that it omits smaller accidents from consideration and thereby reduces the number of datapoints. This makes a statistical approach difficult and minimizes the scale’s predictive power.

A year ago, Hsü² proposed a multi-level scale based on a magnitude/frequency relationship developed originally to predict the probability of natural catastrophes ranging from simple landslides affecting only a small area to impacts of 10-km-diameter meteorites (or comets) capable of significantly altering the Earth’s biota³. Hsü also pointed out that if the radioactivity released is used to express the magnitude of an accident, many smaller, non-polluting incidents such as ‘operational disturbances’ or temporary ‘shut-downs’ would be excluded from statistical analyses². This would significantly reduce the number of available datapoints. Because the reliability of statistical methods depends on the availability of sufficient observational data, any filter that rejects too many datapoints, including Madelin’s scale, is unlikely to be as useful for predictive (and therefore practical) purposes as Hsü’s scale which is based on the amount of money lost (or the extent of damage expressed in monetary units) during an incident. As Hsü points out, for the smallest incidents, the financial losses (involving, for example, only the monetary equivalent of the electricity not produced while the plant is shut down) provide a datapoint, although no pollution need occur. For accidents involving minimal radioactive pollution, Hsü suggests that the potential of future occurrences of cancer should also be considered. Loss of lives could be quantified by using insurance premiums or damage payments in law suits.

The big advantage of Hsü’s scale over Madelin’s is that it makes it possible to add numerous points to our databank to render a statistical assessment of nuclear accident probabilities possible and reliable. As Hsü himself points out^{2,3}, the success of the method is well-known in the earth sciences and has been employed for some time in predicting the probability of the occurrence (repeat time) of earth-

quakes. In the light of such a statistical treatment of natural events (including catastrophes), for example, the old debate between uniformitarian and catastrophist schools seems futile unless the magnitude of events considered is specified.

When potential long-term dangers of fossil fuels, such as a catastrophic increase in the CO₂ content of the terrestrial atmosphere (ref. 4, fig. 9-3) are seriously debated and by some considered to be perhaps greater than the risks entailed by nuclear energy⁵, it is imperative that a scale such as that of Hsü with a powerful prediction potential to foretell realistically the likelihood of nuclear accidents is employed by all organizations having the relevant data and that the results be submitted to the public. Only then can we discard imaginary fears and over-optimistic hopes and lay the foundations of a realistic energy policy for the future.

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“Artificial” HIV?

SIR—Fukasawa and colleagues (*Nature* **333**, 457–461; 1988) suggest that descendants of the ancestral human immunodeficiency virus/simian immunodeficiency virus (HIV/SIVs) “diverged gradually in concert with the evolution of primates, resulting in the occurrence of species-specific viruses in primates”. Although they acknowledge that there may have been some interspecies transmission, the essence of their hypothesis is that there has been species specificity of HIV/SIVs in primates for a long time.

Although this conclusion is consistent with the hypothesis that most primate lentiviruses in existence today are species-specific, and diverged gradually over many millions of years, it does not seem to apply to all of them. If HIV-1 and HIV-2 have diverged during human evolution, why is the nucleotide sequence of HIV-2 much closer to SIV_{MAC} than to HIV-1? Undoubtedly there has been species specificity of SIVs in nature for a long time, but why has SIV_{MAC} never been found in macaques in the wild — only in primate research colonies?

Where are the two lost tribes that kept themselves and their viruses isolated from the rest of mankind for hundreds of thousands of years until the 1960s? An ancient virus of humans which makes people persistently infectious for life, and spreads

as rapidly as HIV-1 has spread in Africa, the United States and Europe in the past decade, would inevitably have spread throughout mankind long ago. The proper place for the lost tribe theory would seem to be in a science fiction novel such as Conan Doyle’s *The Lost World*, rather than in *Nature*. The hypothesis of Smith and colleagues (*Nature* **333**, 573–575; 1988) that HIV-1 may have evolved by natural selection from its common ancestor with HIV-2 as recently as 40 years ago is much more consistent with experimental and epidemiological evidence.

All molecular biologists seem to take it as axiomatic — at least in public — that HIV-1 must have evolved entirely by natural selection. Why? If it is possible that the pathogenic primate lentiviruses (HIV-1, HIV-2 and SIV_{MAC}) could have evolved naturally from a common ancestor since 1948, it must also be possible for them to have evolved by artificial selection from the same common ancestor starting even more recently.

The fact that it is possible, indeed quite simple, for HIV-1 and HIV-2 to have been created by artificial selection, and the AIDS epidemic started deliberately, does not necessarily mean that this actually happened. But it is a possibility that deserves serious critical analysis by the scientific community — in public.

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Too much secrecy

SIR—A remarkable feature of the present system of anonymous refereeing in scientific publications is that it appears to give the referees power without responsibility. It is unprecedented in any other public human activity to have a reviewing body with whom the protagonist cannot directly dispute in front of his ultimate audience. Contrast this with a typical seminar or conference presentation: any questioner can openly raise his point with the author. The author’s response must likewise be open and free from personal innuendos, evasions and irrelevancies. In sport, the umpires must give their impartial verdicts in full view of millions. Travesties are obviously found out. In the judicial process (in civilized countries), a citizen has a right to hear the charges and to refute them openly.

Yet, what do we have at the moment? A system set up, almost as if by design, to propagate COWDUNG (conventional wisdom of the now-dominant group, to quote C.H. Waddington). The deplorable but real tendency of some to hide behind anonymity to write an incompetent, or worse, a motivated report can be inhibited only if the system is changed. Unlike umpiring in sport, there is a

powerful conflict of interest inherent in scientific refereeing, as a referee's own work is often the target of an author's article.

We scientists owe it to ourselves and to our financial patrons to be more open and equitable in the critical assessments of non-consensual views and results. Should we perhaps consider a system of completely open refereeing in which the author is allowed to publish his article together with the signed reports of the referees (if the latter are critical) if he so wished? There would still be some people who might publish and risk their reputations despite the openly stated criticism of experts. This can, however, do no harm in the long run as only truly invariant results and concepts would stand up to the relentless scrutiny by many independent researchers. Popperian falsification tests would consign the failures to the scrap heap anyway.

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Ethical bounds

SIR—In a recent issue (*Nature* 334, 560; 1988), members of the Department of Cell Biology of the Max Planck Institute in Göttingen express the opinion that our study on the activation of the human embryonic genome (*Nature* 332, 459–461; 1988) “oversteps the ethical bounds of scientific work”. They also propose a moratorium on work on human pre-embryos until ethical boundaries have been established.

In the United Kingdom, ethical boundaries for work on human pre-embryos and a regulating authority to supervise them were established in 1985¹. A proposal for the research work reported by us was submitted to this licensing authority and was approved and funded by the Medical Research Council of Great Britain. These circumstances were acknowledged at the end of our letter.

We have each considered the ethical issues raised by our work carefully over many years, and after discussions with theologians, lawyers and philosophers not only believe this work to be ethical but, in common with many respected scientists in this country², believe that limited and controlled systematic research is needed and should be encouraged.

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Outlandish claims

SIR—It would be a pity if a journal such as *Nature* could not find time and space for a serious appraisal of such claims as those of Benveniste and his colleagues (*Nature* 333, 816–818; 1988). But, in my opinion, it is absolutely essential that in the first instance outlandish claims should be assessed critically by means of the most scientifically valid and relevant laboratory approaches available in that field at that time.

Let me give you an example, also from the allergy field, of which I have personal experience and which (needless to say) is frequently subject to such claims. A few years ago a reporter from the local paper (*The Birmingham Post*) sought my opinion about a story that he had picked up in Malvern, that hay-fever sufferers there were being cured by eating beeswax capping obtained from a local bee-keeper. It seemed to me just possible that such people were imbibing small amounts of pollen products within the beeswax, and this was leading to their auto-desensitization. But the problem with this explanation was that their hay fever was caused by hypersensitivity to common grass and tree pollens, whereas the bees were collecting flower pollen. Nevertheless, we set up a small pilot study in our laboratory to explore this possibility, involving the monitoring of levels of antibody (sensitizing and protecting) in the allergic individuals' circulation in relation to any change in their clinical status. This preliminary study led to a full-scale 'double-blind' investigation of the similarly claimed efficacy of pollen B tablets (available over the counter from healthfood shops and chemists) in the treatment of hay fever.

In neither study did we find any experimental evidence, based on our current understanding of the mechanism underlying the immunopathology of allergic responses of the hay-fever type, to suggest that such forms of self-treatment were having any beneficial organic effect. But we had at least made the effort to investigate the claims in a rational scientific manner. Similarly, it would be possible to appraise, for example, the extravagant claims of certain clinical ecologists that over 90 per cent of cases of rheumatoid arthritis are attributable to a food allergy, because a whole battery of laboratory methods of assessing the onset and progression of this disease are now readily available.

Turning to the surprising claims of Benveniste and his colleagues, surely here too it is first necessary to assess them by the most reliable and relevant laboratory procedures currently available in this area. This means that, for a start, one would choose the mast cell rather than the basophil as the effector cell in question because mast cells can be obtained in

highly pure form in high yield, and one would therefore expect much less inter-experimental variation. Furthermore, one would measure the release of histamine (or some other mediator), while at the same time measuring the release of a cytoplasmic marker enzyme such as LDH, to ensure that the effect observed was non-cytolytic and therefore really attributable to the immunological triggering of the effector cell and not due to some artefact, rather than continue to struggle with the attempted measurement of the notoriously unreliable degranulation endpoint.

It is because this technique is so unreliable that the so-called new microscopic test for detecting allergies of the hay fever/asthma type based on this principle (reported by Shelley and Juhlin in *Nature* 191, 1056; 1961) never caught on in routine clinical immunology laboratories. Despite this, Benveniste has persisted in developing a more elaborate assay kit which, in the experience of those of us who have had the opportunity to assess it critically, appears to suffer from many of the experimental shortcomings of the attempted accurate quantitation of basophil degranulation referred to in the report that followed your recent visit to Benveniste's laboratory. The explanations for this experimental variation are manifold: basophils are extremely fragile cells, readily degranulated by nonspecific stimuli; they are present as only a minor population of peripheral blood leukocytes; the amount of IgE antibody bound to their Fc receptors differs from individual to individual, and so forth.

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SIR—While I am highly sceptical of the claims made in the article by Benveniste and his colleagues, the work was nonetheless appropriately conducted with seemingly reasonable controls and reported in scientific fashion. There may well have been errors in methods and/or interpretation as regards the effectiveness of their dispersion procedures, but the scientific community has well-tested standard methods for investigating such claims. These methods do not require the use of journalists or magicians and their inclusion on an investigating team is demeaning to the scientific process. It is also highly unusual to include a specialist in 'misconduct in science' when there have been no allegations of such.

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The stimulation of the fifth force

Nearly three years of ingenious searching may not yet have uncovered evidence that the fifth force, a kind of correction of newtonian gravity, is real, but the search itself has been rewarding.

THE reality or otherwise of the fifth force, the supposed short-range correction to newtonian gravitation, may still be an open question, but there is little doubt that the search for evidence mounted in the past three years has been extraordinarily stimulating. Both experimentalists and theoreticians have done wonders of ingenuity. The flurry of excitement shows vividly how the publication of arresting inferences from intriguing data can have a value going beyond the interest of the original claims. No doubt it is a necessary condition for this benefit that the data should be convincing and the inferences made from them inherently plausible, conditions amply satisfied by Fischbach's re-examination of the Eötvös data of the 1920s.

On balance, the experimentalists seem to have responded the more ingeniously to the challenge of the fifth force. There have been novel designs of torsion balances and, more particularly, novel ways of placing them near perturbing masses, on cliff faces or on the edges of dry docks, for example. But a measurement now reported by C.C. Speake and T.J. Quinn, from the International Bureau of Weights and Measures in Paris (*Phys. Rev. Lett.* **61**, 1340; 1988) seems to point the way to more sensitive measurements of the gravitational attraction between masses separated by laboratory distances. That could be important because Newton's constant of gravitation, G , is one of the least accurately known of the fundamental constants.

Speake and Quinn have been operating on a huge scale, at least by the standards of most precision measurements, having arranged to measure the gravitational attraction between objects of different composition (lead, carbon and copper), each of mass 2–3 kg, and moveable attracting objects with mass no less than 1–78 tonnes (and made, alternately, of lead and brass). As the fifth force is supposed to depend on the composition of the attracting materials, it is necessary to be able to ring changes such as are made possible by this array of materials.

The alternative attracting masses apparently consist of motorized trolleys that can be trundled into position beneath a sealed tank containing the balance. The balance itself consists of a rigid beam with equal arms. The novelty is the suspension for the beam, which pivots on flexible strips rather than a knife-edge, thus avoiding the familiar problems in precision

measurement of knowing just what allowance to make for the elasticity of a supposedly rigid support.

The enclosing tank is first evacuated and then filled with nitrogen at roughly atmospheric pressure, chiefly so as to damp oscillations of the balance. Test objects are weighed under the influence of one or other of the attracting masses. To minimize the effects of geometrical shape, the trolleys made of lead (in reality, there are two each, each carrying nearly half a tonne) consist of layers of lead interspersed with wood to give them nearly the same geometrical shape. Similarly, the 2–3 kg test masses are enclosed in stainless-steel cans of nearly equal shape so as to reduce the corrections due to buoyancy. There is a system of gimbals to ensure that masses can be interchanged accurately (and automatically) on the balance pans.

In essence, the measurement is a null measurement: the balance beam is kept horizontal by a servo-system, regulating electrical currents passed through two coils interacting with two magnets mounted at each end of the beam. Known sources of vibration are reduced as far as possible by the design and then at least partially excluded by filtering the output from the servo-system.

Even so, the system seems not to have been entirely free from trouble. Outgassing from the stainless-steel cans seems always to have been a problem (accounting for changes of pressure of the order of one per cent an hour). But the most persistent source of uncertainty in four series of measurements seems to have been the difficulty of excluding dynamical changes caused by external sources of heat. The wooden layers interleaved with lead insulated that pair of trolleys more efficiently than the brass trolleys from the laboratory floor, providing a systematic difference with composition that might have trapped less careful investigators. Speake and Quinn estimate that their mass differences have a sensitivity of 1 nanogram, corresponding to a force of 10^{-11} N.

The authors are no doubt right to say that their measurements should be readily capable of improvement, and that a tenfold improvement of sensitivity should be possible by paying more attention to the exclusion of design of the effects of external heat. And the result of what they have done so far? Sadly, the persisting errors are comparable with the mean values, which is another way of saying that they

are not significant. The authors claim that they can only exclude the possibility that the fifth force at short distances is greater than about one per cent of ordinary gravitation, which of itself does not do much to advance the cause.

Much the same has emerged from an analysis of past measurements of the position of planetary orbits conducted by C. Talmadge from Purdue University and three colleagues at the Jet Propulsion Laboratory at Pasadena, California. The argument is simple: if the force between a planet and the Sun is not a simple inverse function of the square of the distance, Kepler's Third Law (relating orbital period to semi-major axis and, crucially, in which the mass of the planet does not enter) should not be strictly correct. In practice, Talmadge and his colleagues say, Kepler's Law is rarely verified directly; people measure the orbital period of a planet directly, then calculate the semi-major axis from the supposed value of the constant in the equation — the product of the solar mass and the gravitational constant.

The group seizes the opportunity of using accurate data for the positions of planets derived from sighting shots by passing spacecraft as well as from the longer series of data based on radar-ranging measurements of the objects of the Solar System. Evidently there is a substantial volume of data not fully made use of in previous analyses. One of the unexpected byproducts of the exercise is the discovery that the data can be made to yield more accurate estimates of the anomalous rates of precession of the orbits of the planets out to and including Jupiter.

But, sadly, the outcome for the main purpose of the calculation is again disappointing. From information about the distance of the orbit of a particular planet, it is obviously possible to obtain information about the strength of the fifth force in some region spanned by the average position of the orbit. Sadly, again, the estimated errors of the estimates Talmadge and his colleagues have derived are comparable with, or greater than, the estimates themselves, so that only extreme values of the parameters defining the fifth force (if there is one) can be confidently excluded. But, in a sense, "So what?". Observers will remark that the hunt for this still elusive phenomenon has already been worthwhile, whatever the eventual outcome.

John Maddox

Nonlinear dynamics

Yin–yang and the art of noise

Ian Stewart

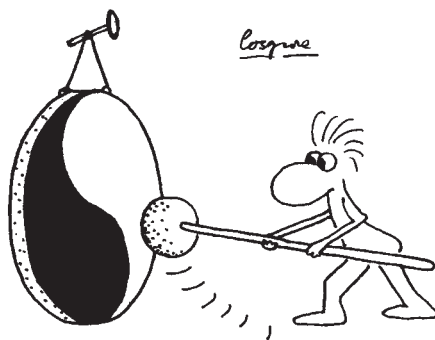
DYNAMICAL systems can do rather complicated things. Much of the recent progress in nonlinear dynamics has come from the recognition that some of these things are more common than others. This idea is formalized in the concept of genericity: a property is generic if it is 'almost always' true. The hope is that generic systems are sufficiently well behaved to be classified in some useful qualitative manner. A decade ago this was called the *yin–yang* problem: find systems simple enough to be classified (*yin*) but complicated enough to be generic (*yang*). The fact that *yin* and *yang* are opposed polarities indicated the difficulties of the problem; but their ultimate unity symbolized the hope that these difficulties could be overcome. For a time this hope rested on the concept of structural stability, a kind of dynamical robustness, but subtle counter examples shattered it. Now, Christopher Zeeman proposes a modified notion of structural stability, for which the *yin–yang* problem can be solved (*Nonlinearity* 1, 115–155; 1988). The ideas are closely related to experimental technique, but go rather against the grain of conventional thinking about dynamical systems. Their novelty lies not so much in their content — which is in many respects classical — but in the manner of their application.

A drop of ink placed gently into a container of fluid slowly spreads under the influence of random molecular collisions. Nevertheless this has a well defined deterministic mathematical structure, because the density distribution of the ink averages out the stochastic effects, and its evolution is well modelled by a deterministic partial differential equation. The flow of heat obeys a similar equation for similar reasons.

Noisy fluid

Analytically, a dynamical system is specified by a differential equation which can be written in the form $dx = f(x)dt$ for some function f . Geometrically, its solutions can be thought of as a flow, where initial points swirl through phase space (the space of states x) like some hypothetical fluid. All this is perfectly deterministic. But now suppose that the fluid is noisy, so that flow is disturbed by random agitation. Now we have a stochastic dynamical system, $dx = f(x)dt + \varepsilon dw$, with noise level ε and an explicit random term dw . The statistics of dw can be specified at will, but a Wiener process, or 'white noise', has certain technical advantages.

In such a system, the trajectories of individual points are highly irregular, and it is better to think of their average behaviour. Imagine that some cluster of points is coloured with ink. The density of the ink may not be uniform: the colour may be darker in some regions than in others. If the entire cluster obeys the noisy dynamic, the averaged effects of the random noise act like diffusion. The cluster combines a deterministic motion given by the original flow with a diffusion process



driven by the noise; the ink smears out into a fuzzy but structured pattern.

Although the behaviour of individual points is now random, that of the entire cluster remains deterministic. This is not a paradox: the whole point about statistical averages is that they smear out random effects. The evolution of the density distribution is prescribed by a deterministic partial differential equation, the Fokker–Planck diffusion equation. All this has been understood for a long time and is of considerable practical importance: it applies to problems ranging from the spread of bacterial cultures to the control of cruise missiles.

Suppose now that the phase space of the system, the set of possible states x , is a compact manifold — a smooth, multi-dimensional space of bounded size. Then an initial distribution cannot diffuse away to nothing, so it has to do something more interesting. Often it settles to a stationary distribution, a probabilistic steady state. Intuitively, if you wait long enough for the system to settle down, so that it can 'explore' the whole of phase space, then the probability of finding it in a given region will settle down to a unique value.

Zeeman's idea is that this unique steady state is a fundamental invariant of the original deterministic system. That is, associated with any dynamical system, there is a unique family of density distributions, one for each noise level ε . To obtain them, add noise εdw to the

deterministic dynamic $f(x)dt$ and solve the resulting Fokker–Planck equation. In some sense these distributions provide increasingly accurate 'smoothed versions' of the long-term dynamics. Because experimental systems always have noise, it can be argued that in some respects the noisy system should resemble the observed behaviour better than the original deterministic system.

Steady state

The paper contains several important technical contributions on properties of the Fokker–Planck equation. In particular there is a new theorem on the existence, uniqueness and global attractiveness of the steady-state distribution, whose proof — remarkably — revives the classical methods of Otto Perron and Georg Frobenius in a new context. Zeeman also presents many simple examples to illustrate the sometimes surprising behaviour of the steady-state distribution and provides general intuition. For example, consider a stable limit cycle in the plane, where the deterministic dynamics is periodic. The corresponding steady-state distribution has a graph whose form resembles a volcanic crater: a roughly circular ridge whose peak lies over the limit cycle. But the ridge varies in height according to the speed with which the particle moves round the cycle. The main focus of the paper, however, is that there is a natural generic property associated with the steady-state distribution.

Qualitatively, the important features of the steady-state distribution are its critical points, where the derivative vanishes. These include, in particular, the places where the density is at a local maximum or minimum. There is a well established notion of a 'nondegenerate' critical point: a place where the behaviour is qualitatively determined by the quadratic part of the Taylor series, $f(x) \approx f(x_0) + (x - x_0)f'(x - x_0) + \frac{1}{2}(x - x_0)^T f''(x - x_0)$. . . Zeeman shows that the property "all critical points of the steady-state distribution are nondegenerate" is generic. Furthermore, generic systems can be classified completely, using the methods developed by René Thom, John Mather and Vladimir Arnold a decade ago and variously known as catastrophe or singularity theory.

The results also suggest several interesting lines of research, marrying the well developed theory of critical points and the theories of dynamical systems and diffusion. The underlying concepts may be well established, but the novelty lies in the way several powerful techniques combine together to create a new point of view on qualitative dynamics. Sometimes the key to progress is to change the question. □

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Gene therapy

Intracellular immunization

David Baltimore

In an article published in *Nature* last year (329, 219–222; 1987), Ira Herskowitz pointed out that a good way to study the function of a cloned gene is to generate mutants that confer a dominant negative phenotype. When a plasmid expressing such a mutant gene is established stably in cells, its product can dominantly interfere with a function performed by the product of the resident gene, thus helping to illuminate the function of the wild-type allele. Now, Alan Friedman, Steven Triezenberg and Steven McKnight in this issue (*Nature* 335, 452–454; 1988) have extended this strategy to viral resistance by implanting in cells a gene encoding a fragment of a herpesvirus protein, VP16.

The wild-type VP16 activates transcription of herpesvirus 'immediate early' genes and sets in motion the whole viral transcriptional programme. Friedman *et al.* have generated a fragment that lacks that part of the protein which activates transcription but retains regions thought to be responsible for interacting with cellular DNA binding proteins. In theory, the endogenously produced fragment should compete with the protein made by an infecting herpesvirus for binding to cellular proteins and thus prevent the virus from activating transcription of its genes. The theory is borne out by practice — the cells expressing the fragment are remarkably well protected from infection. It must be assumed that other lines expressing the fragment would also be protected.

These cells have been genetically engineered to be virus-resistant, but is this an *in vitro* gimmick or is it a model for a new form of therapy? Friedman *et al.* clearly believe that it can be translated into a clinically useful procedure which can be extended to humans. They explicitly suggest that such a strategy might work for HIV, the retrovirus that causes AIDS.

At first blush it seems hard to think of protecting an individual against an infection by genetically engineering resistance into the person's cells. The obstacles are formidable but, taking HIV as an example, we can imagine what would be involved.

First, what cells could be protected in this manner? In principle, any cells that are easily renewed from available precursors could be protected if genes can be implanted into their precursors. In practice, blood cells are the primary target because they are continuously renewed from bone marrow stem cells. Luckily, HIV is largely an infection of blood cells,

T lymphocytes and monocytes/macrophages. These cells derive from haematopoietic stem cells whose purification, at least in the mouse, has recently been achieved (Spangrude G.J. *et al.* *Science* 241, 58–62; 1988). Thus, the cells are available and several groups interested in the related goal of gene therapy have shown that they can be engineered to express new genes introduced using retrovirus vectors (Wetherall, D.J. *Nature* 331, 13–14; 1988). Such a procedure could be adapted to make a retrovirus fight a retrovirus. Let us carry through the idea that cells can be protected in this manner.

The procedure would be initiated by taking from an HIV-infected individual bone-marrow cells, including the all-important haematopoietic stem cells. Using either the mixed population or a purified fraction, the stem cells must be infected or transfected with a virus or DNA construct that encodes an RNA or protein able dominantly to interfere with the

intracellular growth of HIV. This could take many forms; an RNA that binds to a regulatory protein (if such RNA-protein interactions occur); an anti-sense RNA; a mutant form of some viral polypeptide that forms multimers, the mutant being able to interfere with the multimer's function; a DNA-binding protein that has had its regulatory region amputated, and so on. The amount of this protein that needs to be made could be relatively small, but the transcriptional control elements must be ones that can function when the stem cell matures to T cells or monocyte/macrophages. Designing such a dominant interfering molecule may not be as easy for other cases as it was for VP16 where a clear division of labour was evident in the molecule. Further, the fragment must be non-toxic.

The modified stem cells must then be injected into the patient and home back to the marrow. To give space for the growth of the implanted cells, the marrow could be partially cleared by irradiation or with a cytotoxic drug. If the strategy is to succeed the protected cell lineage must then preferentially flourish; but perhaps its progeny would be at a selective advantage by virtue of their resistance to HIV infection.

I believe that this type of procedure has



In 1877, Charles Darwin wrote a book called *The Different Forms of Flowers on Plants of the Same Species* in which he puzzled over the evolution of the reproductive strategies used by plants. One problem was the evolution of plants with separate sexes (dioecious plants) from hermaphrodite ancestors — "There is much difficulty in understanding why hermaphrodite plants should ever have been rendered dioecious". This problem has led to much theoretical work, and it is now widely believed that an initial stage in the evolution of full dioecy may have been the evolution and persistence of females in otherwise hermaphrodite populations. The picture shows male (left) and female (right) flowers of the buffalo gourd, *Cucurbita foetidissima*. In natural populations, some plants produce both flower types whereas others produce only female flowers. On page 431 of this issue, Joshua Kohn shows that in this species seeds from females survive their first year in nature more than twice as often as seeds from hermaphrodites. This may explain why female plants are maintained in the otherwise hermaphrodite population.

Rory Howlett

a real chance of success, and propose that it be called intracellular immunization. It is a true immunization process because if it works the patient becomes immune to HIV infection. But it is not likely to be used for uninfected individuals because of its complexity, possible side-effects and the absence of possible stem-cell selection in an uninfected individual. It would probably be used on an already infected person where its purpose would be therapeutic. But it is still, at the cellular level, a form of immunization, not a therapeutic attack on the virus.

Will intracellular immunization actually work? I see no theoretical barriers, only practical questions. The practical problems are similar to those posed by gene therapy and are being actively examined in many laboratories (for example, Dzierzak, E.A. *et al.* *Nature* **331**, 35–41; 1988). The intracellular immunization protocol is equivalent to a bone-marrow transplant, a widely used technique. Such transplants have a high mortality because they usually involve implants of a partially matched donor's cells into the recipient. Here the transplant would be autologous, avoiding the problems of immunological incompatibility.

The open questions, however, are still serious ones. What is a good dominant inhibitory product? What is a good expression system? Moreover, even if enough cells can be transformed with an effective inhibitor, there is no guarantee that the protected clone will be sufficiently well selected to dominate the body's haematopoietic system, although we could imagine a co-expressed, selectable gene to overcome this difficulty.

A conceivable but difficult variant of this procedure would be to use a retrovirus, carrying a dominant protective gene, that could be directly injected into an individual. If such a virus would home to marrow cells because of a surface protein that was stem-cell tropic, it could be a route to direct therapy — or conceivably, prevention — with no intermediate removal and reimplanting of cells.

Given the difficulty of standard immunization against HIV and the problems of designing good therapeutic agents, I believe intracellular immunization has as good a chance as any other procedure of becoming a real AIDS therapy. Friedman *et al.* have now demonstrated that at the cellular level a dominant inhibitory protein can be protective. It now seems realistic to attempt the next steps, to animals and to HIV. HIV may, in fact, be a uniquely suitable target because it grows largely, perhaps solely, in haematopoietic cells. Even partial protection against HIV might be valuable. □

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Oxide superconductors

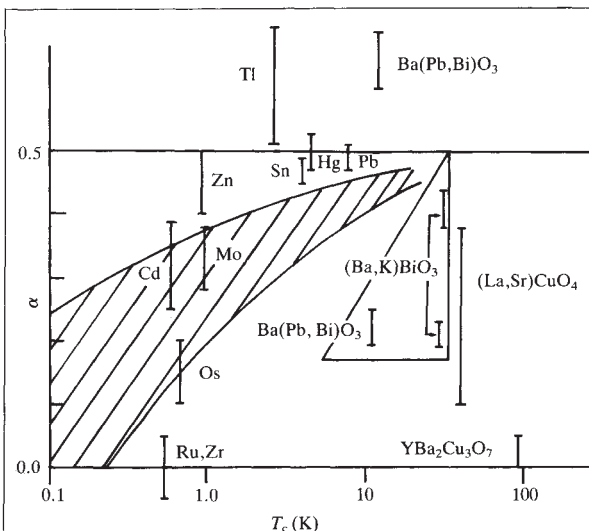
Isotope shift controversies

Philip B. Allen

THE high transition temperatures (T_c) of copper-oxide superconductors continue to resist explanation. Theories abound, but decisive experimental tests do not. Valuable clues should be available from measurements of the dependence of T_c on the isotope content of samples. This is because many theories invoke lattice vibrations as a fundamental ingredient of superconductivity, and the frequencies of

mass M_i : $\alpha_i = -d \log T_c / d \log M_i$. If the partial shifts α_i are all positive and add up to a value near 1/2, this again indicates a conventional mechanism.

The isotope shift was discovered¹ in 1950, well before the seminal theory⁴ of Bardeen, Cooper and Schrieffer (BCS). The measured value³, $\alpha \approx 0.50$ for mercury, was a great surprise. It had been known since Bloch's work⁵ of 1928 that an 'independent electron' model accounts well for ordinary metallic conductivity but cannot account for superconductivity. Thus electrons must be correlated, not independent, for superconductivity to occur. The most obvious interaction to cause this correlated behaviour is the strong repulsive Coulomb interaction between like (negative) charges. But this has no dependence on the nuclear isotope mass. The value $\alpha \approx 1/2$ shows that atomic vibrations must be a prime cause of the superconducting correlations. When vibrations are described by quantum theory, they behave like particles, called phonons, which can interact with electrons. This electron-phonon interaction is for most purposes fairly weak compared to direct



Isotope shift α plotted against transition temperature T_c on a log scale. The value $\alpha=1/2$ occurs if electron-phonon interactions are the only active mechanism. Hatching, the expected range of α assuming $\Theta_D \approx 200$ K and the Coulomb repulsive parameter $\mu^* = 0.10$ – 0.16 . Data for elemental superconductors⁶ can be explained by standard theory. Data for oxide superconductors¹ are unexplained. The new results of Hinks *et al.*² and Batlogg *et al.*² are in the triangular region.

these are sensitive to atomic masses. On page 419 of this issue¹, Hinks *et al.* report that the 30-K oxide superconductor $(\text{Ba,K})\text{BiO}_3$ is significantly affected by the oxygen isotope content. But their data and interpretation that the material is a conventional superconductor are challenged in a paper by Batlogg *et al.*² to be published in the next issue of *Physical Review Letters*.

In superconductors consisting of a single metallic element (such as lead or niobium), T_c scales with isotope mass M as $M^{-\alpha}$ where the exponent α is the isotope shift. Values of α near 1/2 indicate strongly that a conventional electron-phonon mechanism causes the superconductivity. Most other mechanisms are expected to be independent of isotope mass: $\alpha=0$. Although permitted theoretically, other mechanisms have never been unambiguously identified in any metallic superconductor. For compound superconductors, the corresponding parameter is the partial isotope shift α_i of element i of

Coulomb interactions.

It is now well understood how superconductivity is normally caused by a combination of two factors: the time-dependence of the vibrations; and the characteristic energy spectrum of electrons in a metal, with empty states arbitrarily close in energy but sharply separated from filled states. As the temperature increases, the sharp separation smears out, and eventually superconductivity is destroyed at T_c . In BCS theory, $T_c \sim \Theta_D \exp(-1/\lambda_{\text{eff}})$. The interaction parameter λ_{eff} depends only weakly on isotope mass, but the Debye temperature Θ_D , which characterizes the vibrational frequencies, varies as $M^{-1/2}$ yielding $\alpha \approx 1/2$.

This electron-phonon mechanism is now universally accepted as a dominant cause of superconductivity in most known superconductors. Ironically, the isotope shift which provided the original proof of this mechanism is now one of the least well explained properties. The figure above shows measured values⁶ of α and

theoretical expectations. There is at least no serious contradiction for elemental superconductors between the measured deviations of α from $1/2$ and theory, but quantitative comparisons are not easy. Theory and experiment agree that for conventional superconductors α becomes closer to $1/2$ as T_c becomes larger. This trend is violated by the new copper-oxide-based superconductors, however, as also shown in the figure. Thus we have fairly strong presumptive evidence for a new mechanism at work.

Physicists have never abandoned the hope that the stronger Coulomb interaction could be recruited to help produce the pair correlations that cause superconductivity. This would have the effect of replacing Θ_0 in the BCS equation by a larger number Θ_{eff} which would have a reduced dependence on M . This would also permit larger values of T_c . As can be seen from the figure both the larger values of T_c and the reduced partial shifts α_{ox} in copper-oxide-based superconductors seem to require an additional mechanism of this type. But as suggested by Hinks *et al.* in this issue¹, the case is not airtight.

A sobering example is the case^{7,8} of palladium hydride for which the replacement of hydrogen by its heavier isotope deuterium has the reverse effect of raising T_c . That is, α_{H} is negative even though T_c is only about 11 K. This lies clearly outside the realm of BCS-like behaviour. Nevertheless, a phonon mechanism probably does dominate and the peculiar value of α_{H} has been ascribed⁹ to other causes — anharmonicity and 'zero-point' shifts of energy bands. Thus the strongest reason for belief that an unconventional mechanism operates in the copper-oxide superconductors is their high T_c . But the belief that T_c of conventional superconductors is limited to low value has no rigorous basis and rests largely on the observed difficulty of getting $T_c > 23$ K.

Thus the discovery¹⁰ of $T_c \approx 30$ K in the non-copper-bearing oxide $(\text{Ba,K})\text{BiO}_3$ has opened a new chapter. Hinks *et al.*¹ claim strong evidence on the basis of their value $\alpha_{\text{ox}} \approx 0.4$ for a conventional mechanism. They observe that a similar value, $\alpha_{\text{ox}} \approx 0.6$ was reported¹¹ for the closely related compound $\text{Ba}(\text{Pb,Bi})\text{O}_3$ ($T_c \approx 12$ K). If these results are accepted, the limits to T_c of conventional superconductors are higher than previously suspected, and the case for an unconventional

mechanism in the copper-oxide materials is less compelling.

Batlogg *et al.*² report a somewhat different value, $\alpha_{\text{ox}} \approx 0.2$, for both $(\text{Ba,K})\text{BiO}_3$ and $\text{Ba}(\text{Pb,Bi})\text{O}_3$. This value can be consistent or inconsistent with conventional theory depending on whether or not the other atoms (such as Bi) have significant partial shifts. Batlogg *et al.* cite independent experiments to argue that the mechanism is unconventional. But because even their value is clearly different from zero, the unconventional mechanism has to be one where phonons are a factor, either secondary or parasitic. The source of the discrepancy between the various values of α_{ox} is not known. Many factors can enter during the various annealing cycles used to interchange ^{18}O and ^{16}O , such as purity of the gases, lack of control over where the new oxygen atoms settle in the crystal lattice

and difficulties in measuring the degree of isotope exchange accomplished.

It is clear that no single inference can be unambiguously drawn. The most widely held belief seems to be that a gradual crossover is occurring between conventional and unconventional mechanisms. The most popular unconventional mechanisms for the copper-oxide materials are based on magnetic aspects of the Coulomb interactions. These seem¹² to be absent in the non-copper-bearing bismuth-oxide systems, however, making any interpretation of the figure an almost completely arbitrary perception in the eye of the beholder. Independent rigorous repetitions of these isotope experiments are needed to resolve the differences and permit interpretation. □

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Galactic evolution

Biography of the galaxies

M. G. Edmunds

EVOLUTION implies change. But whereas in biology, evolution occurs through many generations of individuals, in astronomy the 'individuals' — the galaxies — belong to the only generation and could be almost as old as the Universe itself. How they came to be, how they have developed and how their mutual interactions have changed their life histories were discussed at a recent, wide-ranging meeting*.

Because of the time it takes light from distant galaxies to reach us, we see them as they were in a previous epoch. Thus both their age and distance are usually described in terms of their redshift z , the fractional increase in the wavelengths of light they emit caused by the cosmological expansion of the Universe. In these terms, galaxy formation is typically thought to have started at $z = 10$ – 20 (M. Rees, University of Cambridge). It probably continued for a billion (10^9) years and so could still have been occurring at $z \approx 3$.

Quasars have been observed out to distances of $z=4$ and can be used as probes of galactic evolution in space closer to us. Intervening gas clouds at $z=2$ – 3 have been known for some time, their presence deduced from the absorption-line spectra they cause in the bright emission from quasars. But attempts to detect the characteristic emission spectra expected if star formation is occurring in these clouds have been unsuccessful (H. Smith, University of California, San Diego). The extended radio emission from background quasars also could be used, to reveal spatially resolved disk-like 21-cm absorption by hydrogen gas in these intermediate-

redshift clouds if they are forming or about to form galaxies (F. H. Briggs, University of Pittsburgh).

The early evolution of galaxies must depend heavily on the rate at which stars form from such clouds, a process that is poorly understood. The longest timescale in the process seems to be the conversion of atomic to molecular gas (H. Zinnecker, Max Planck Institut für Astronomie). But what really controls the rate of star formation remains uncertain, although the gas- and total-mass densities seem to be important.

The continued evolution of galaxies is reflected by the changing populations of stars within them. Comparisons of local galaxies with galaxies at $z=0.5$ (hence less evolved) show that galaxies were different when roughly half their present age. The complexities of interpreting the integrated spectra of light emitted from distant galaxies by the methods of population synthesis were well demonstrated at the meeting. But the fainter elliptical galaxies in intermediate-redshift clusters do show evidence of star formation having occurred more recently in them than in the older galaxies we see locally (A. Pickles, Kapteyn Laboratory). But it seems that all (or nearly all) galaxies include stellar components that date back at least 10^{10} years.

The cumulative effects of supernova explosions of stars can lead to systematic outflows of gas or even to oscillatory 'bursting' of star formation (N. Arimoto, University of Heidelberg). Such winds could fall back into a galaxy, with important consequences for the evolution of the interstellar medium. ►

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* *Evolutionary Phenomena in Galaxies*, Tenerife, 4–15 July 1988.

The very strong emission of radiation from some galaxies has given rise to much speculation that a massive black hole (10^6 – 10^8 solar masses) can form and grow at the centre, fed by material from the galaxy. As a less exotic alternative, R. Terlevich (Royal Greenwich Observatory) outlined his starburst model for bright, Seyfert nuclei, in which massive stars (but not unreasonably large, only 80–100 solar masses) lose their envelopes by stellar winds to reveal very hot cores.

The hard radiation from these cores is sufficient to produce the line-intensity ratios observed in the emission spectra of Seyfert nuclei. The stellar wind, together with supernova explosions, can produce the large line widths. The supernova explosions could also account for the variability of brightness, even giving some quasiperiodic light echoes if the supernovae occur in a dense interstellar environment.

The model accounts for so many of the observed phenomena of active galactic nuclei without requiring a massive black hole to generate the energy, that it is tempting to see how far the model can be taken. But the properties of quasars, thought to be a class of especially active galactic nuclei, may be just too extreme.

It is also clear now that galactic evolution can be strongly influenced by galaxy–galaxy interactions. R. Joseph (Imperial College, London) showed ample evidence of the violent effects of such interactions which can temporarily increase a hundred-fold the rate of star formation. There may in fact be no such thing as a galaxy that has

formed or evolved in isolation: indeed, estimates of initial sizes and separations indicate that all galaxies may have interacted at $z=1$ – 2 . A large part of the stellar halo of our own Galaxy could perhaps be the result of small satellite galaxies being accreted onto it.

Our ignorance about the star-forming process is not the only hindrance to elucidating galactic evolution. More crucially, observations of the dynamics of galactic motion suggest that we are seeing only a tenth of all the matter in the Universe. Some progress is being made; both G. Gilmore (Cambridge University) and A. Robin (Observatoire de Besançon) reported that there no longer seems to be any need to invoke missing mass in the local region of the disk of our Galaxy. And D. Forbes (Space Telescope Science Institute) deduced from observations of rotation curves in spiral galaxies in clusters either that the galaxies near the core of the cluster are stripped of their individual halos of invisible mass or that they cannot form them in the first place.

It is rather arrogant to discuss galactic evolution when we do not know the nature of 90 per cent of a galaxy's mass. But it is encouraging to recall that biological evolution was recognized long before the molecular basis of genetics had been discovered. Perhaps astronomers will be able to elucidate the general outline of galactic evolution before many of the details are known. □

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Meteorites

Tracing the sources

I. P. Wright and M. M. Grady

THE laboratory study of meteorites continues to help consolidate aspects of astronomical and planetary research. At a recent conference*, meteoriticists discussed, among other subjects, the relationships between meteorites and entities such as asteroids, stars and interstellar dust. In attempting to constrain the origin of meteorites, research effort has been focused on determining the nature of the materials from which they formed and relating the current collection to the respective parent bodies.

Meteorites, representing the major source of extraterrestrial material available for study, provide vital information on the evolution of the Solar System. The elaborate classification, based on their chemistry and petrology, into chondrites and differentiated meteorites such as silicate-rich achondrites and irons, is

ultimately linked to their origins, although unravelling the connection is inevitably tortuous.

It has long been recognized that isotopically anomalous components in meteorites reveal important clues about pre-solar

processes. Recently, the nature of two pre-solar components in CM carbonaceous chondrites, thought to be among the most primitive samples, has been discovered. The first component occurs as 1–5-nm diamonds¹ associated with large enrichments of ^{14}N and the light and heavy isotopes of xenon (Xe-HL component). The diamonds have been variously explained as the products of chemical vapour deposition in a circumstellar shell¹, production in a binary star system² or shock processing of amorphous carbon and graphite^{3,4}. Diamonds have also been found in CI meteorites; 5–10 per cent of the crystallites have twinned structures, which is taken as evidence of a high-pressure origin (P. Buseck, Arizona State University) although twinning in diamonds produced by chemical vapour deposition in the laboratory has been observed⁵.

The second pre-solar carbonaceous component recently identified in primitive carbonaceous chondrites is silicon carbide⁶. Electron microscopy of this material (T. Bernatowicz, Washington University) reveals the complexity of the SiC grains. In addition to cubic (β) SiC, there are other polytypes and various twinning effects. It is hoped that by fully describing the properties of the SiC grains it should be possible to constrain the formation conditions. Meanwhile, ion-probe studies (E. Anders, Chicago University) continue to produce highly informative carbon, nitrogen and silicon isotope data on individual SiC grains. Out of 12 grains analysed in the most recent study, silicon isotope data appear to demand at least seven different sources, perhaps different stars. But the accompanying carbon and nitrogen isotope data are rampant with no apparent correlations: $^{14}\text{N}/^{15}\text{N}$ ratios range from 6,200 to 65 and $^{12}\text{C}/^{13}\text{C}$ from 160 to 11, compared with typical solar values of 272 and 89.

Details of the nucleosynthetic history of these SiC grains remain unexplained, although preliminary results show that grains having silicon isotope ratios close to those of the Solar System have well-formed crystal structures, whereas one

Von dem bonnerstein gefallē jm rcij. iar: vor Ensisheim



Woodcut depicting the fall of the Ensisheim LL chondrite in 1492. (From ref. 8.)

*51st annual meeting of the Meteoritical Society, University of Arkansas, 18–22 July 1988.

grain with an anomalous silicon isotope composition is somewhat rounded and pitted, suggesting that this particular grain was eroded in pre-solar space, by sputtering, collisions and so on. There have been attempts to estimate the pre-solar exposure age of the SiC grains from an appraisal of ^{21}Ne excesses (Anders). The result (about 50×10^6 years) is considerably shorter than the putative lifetime of interstellar grains ($500\text{--}1,000 \times 10^6$ years) suggesting that the Solar System could have formed from rather young material.

Perhaps the most important aspect of meteorite research is to identify the meteorite parent bodies. This has already been done successfully for a few lunar meteorites, the SNC meteorites (which are probably of martian origin) and the eucrites (achondritic meteorites which are thought to come from the asteroid 4 Vesta). But for the ordinary chondrites, which comprise about 70 per cent of all observed meteorite falls, there are apparently no suitable candidates amongst the main-belt asteroids. P. Pellas (Laboratoire de Minéralogie du Muséum and CNRS, Paris) demonstrated that of the xenolithic clasts described in meteorite breccias (which ostensibly form an unbiased sample of main-belt asteroid debris), nearly 40 per cent are ordinary chondrite-like material. To explain this statistically important group of meteorites, Pellas revived an earlier idea⁷ that the type-S asteroids, which have moderate albedos, must be the progenitors of ordinary chondrites.

Ureilites (another group of achondrites) continue to puzzle, having chemical and mineralogical properties which suggest formation by igneous processes, and having oxygen isotopic signatures indicative of nebular effects. Curiously, whereas most meteorites formed about 4.5×10^9 years ago, the ureilites seem to show evidence of a chemical event at less than 3.74×10^9 years ago (C. Goodrich, University of Arizona). This event could be the crystallization age demonstrating the complexity of this group of meteorites.

K. Keil (University of New Mexico) gave insights into the formation conditions of meteorites by demonstrating the potentially complex cooling history of the enstatite achondrites. His results elucidate the conditions prevailing early in the history of the asteroid belt and suggest that, at least in one case, partially molten bodies were fragmented following impacts with other solidified projectiles. □

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Materials science

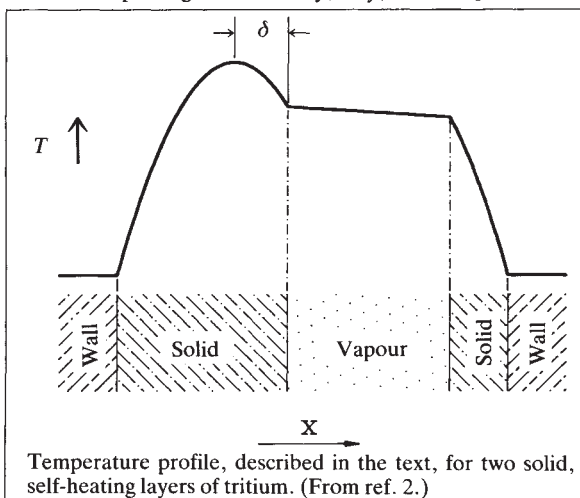
Smooth tritium for laser fusion

Robert W. Cahn

THE Matthew principle is a well founded informal generalization in science and in life: the large will grow larger, the small will vanish. It is named after the disconcerting biblical passage¹ in which St Matthew recounts the parable of the talents: "To every person that has something, even more will be given and he will have more than enough; but the person who has nothing, even the little that he has will be taken away from him". In materials science, the standard exemplar of the Matthew principle is the process termed Ostwald ripening. If an alloy, say, con-

tains a particular precipitate size over smaller and larger ones². But in general, the Matthew principle leads to the enhancement of initial variations and irregularities.

The new study by Hoffer and Foreman² refers to the radioactively induced sublimation in solid tritium. Tritium is radioactive, generating low-energy electrons, which are absorbed within 20 micrometres in solid tritium, depositing heat and ^3He . A slab of solid tritium, the outer surfaces of which are kept at constant temperature, would therefore have a parabolic temperature distribution, with a maximum at



tains a distribution of spherical precipitates of diverse sizes, then, so long as the constituents of the precipitates are soluble in the matrix and can diffuse, the larger spheres will grow and the smaller will shrink, following a well established $t^{1/3}$ dependence on time. In a new study² of a non-metallic system, Hoffer and Foreman demonstrate that there are circumstances under which, contrary to the Matthew principle, initial variations are evened out. They have used radioactive tritium in the study, which has important implications for nuclear physicists exploring the use of frozen tritium pellets for nuclear-fusion power generation.

Ostwald ripening occurs because the solubility of the precipitates is enhanced near a highly curved interface, that is, near a small sphere. The kinetics depend on solubility, diffusivity, the specific energy of the interface and the temperature (see ref. 3). The universality of the Ostwald ripening phenomenon has been denied by the observations of ultrafine particles in certain alloys which do not ripen at all, for reasons which are still disputed, and also by the recent analysis of the role of elastic stress at coherent interfaces, which can result in a preference for

a particular precipitate size over smaller and larger ones⁴. But in general, the Matthew principle leads to the enhancement of initial variations and irregularities. The new study by Hoffer and Foreman² refers to the radioactively induced sublimation in solid tritium. Tritium is radioactive, generating low-energy electrons, which are absorbed within 20 micrometres in solid tritium, depositing heat and ^3He . A slab of solid tritium, the outer surfaces of which are kept at constant temperature, would therefore have a parabolic temperature distribution, with a maximum at the plane in the centre. If a thin slice in such a slab is converted into vapour, so that the original slab now consists of two unequal, thinner slabs (see figure), heat should be transferred across the vapour-filled gap from the thicker slice (where more heat is generated) to the thinner by sublimation. Assuming that there is no obstacle to the flow of vapour, then because the rate of heat generation, $\dot{q}$, in the solid is everywhere uniform, the rate of decrease of the 'excess thickness', δ , should be given by $\delta = \delta_0 \exp(-t/t_{\min})$, where δ_0 is the initial value of δ and $t_{\min} = H_s/\dot{q}$; H_s is the heat of sublimation. The end result should be a pair of slabs of equal thickness.

Hoffer and Foreman tested this prediction experimentally, using a cylindrical geometry. They froze freshly prepared liquid molecular tritium in a horizontal cylindrical vessel (5.74 millimetres deep and across) at constant temperature with end windows: the solid tritium initially has the original meniscus form of the liquid and thus has the shape of a concave lens on the lower side only of the cylinder. Progressively, sublimation converts this into a uniform annular coating of the entire cylindrical wall, the kinetics following the predicted relation, with $t_{\min}$ only a few per cent greater than the predicted value of 14.4 minutes at 19.6 K. When the same experiment is done with 7-day-old tritium, $t_{\min}$ is more than six times greater; this Hoffer and Foreman attribute to the interference with tritium vapour flow caused by the stagnant pool of helium, the radioactive decay product. Sublimation of the self-heated radioactive solid thus leads to removal of an initial geometrical irregularity.

This process is somewhat reminiscent of

the removal of protuberances and grooves from a mechanically polished metal surface by electrolytic polishing. For this, the rate of migration of ions through a thin, viscous electrolyte layer at the metal surface is greater where a protuberance causes local reduction of the thickness of the viscous layer. An analogous process is polishing by simple differential evaporation in a vacuum, where a protuberance evaporates faster than a groove⁵.

These findings are relevant to the design of fuel targets for nuclear-fusion power based on inertial confinement (see the recent News and Views article by R.S. Pease⁶). The demands on the dimensional uniformity and symmetry of such targets are extreme and the difficulties of making them are correspondingly great⁷. A.J. Martin, D. Musinski and R.D. Simms proposed in 1985, in unpublished work cited by Hoffer and Foreman, that the sublimation effect, which they predicted, could be used to make a deuterium-tritium target in the form of a highly uniform, spherical microannulus. The

possibilities are discussed in a forthcoming paper by Hoffer and Foreman⁸; they look promising and this approach would presumably be much less troublesome than others: for example, absorbing a uniform annulus of liquid deuterium-tritium in a porous polymer foam within a microballoon. A fusion target consisting of a frozen, uniform microannulus of deuterium-tritium could also easily withstand the mechanical effects of its rapid insertion into the focus of the initiating lasers. □

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Cancer

Gene losses in human tumours

Bruce Ponder

GENETIC changes which contribute to cancer include altered gene function, altered expression of genes, or their loss (Table 1). For some years, mutations of dominantly acting oncogenes have been the centre of attention; but the recent description of the genetic events in the development of retinoblastoma has focused interest once again on losses of gene function. A report by Vogelstein *et al.*¹ of multiple genetic losses in colonic cancers is the latest in a series of such findings; other examples are given in Table 2.

Retinoblastoma

Almost 20 years ago, De Mars proposed² that in certain familial cancers, gene carriers might be heterozygous for a recessive mutation, and that the cancer appears because of subsequent somatic mutation causing an individual cell to become homozygous for the cancer-causing gene. Subsequently, Knudson's analysis³ of the age-specific incidence of familial and sporadic forms of retinoblastoma and other childhood cancers led him to suggest that development of these cancers requires two events (or 'hits'): he too proposed that in hereditary cancers the first event is provided in the germ line, whereas in non-hereditary cancers both have to occur within the same somatic cell.

In the past few years, these predictions have been confirmed at the molecular level. The development of retinoblastoma has been shown to involve loss of both

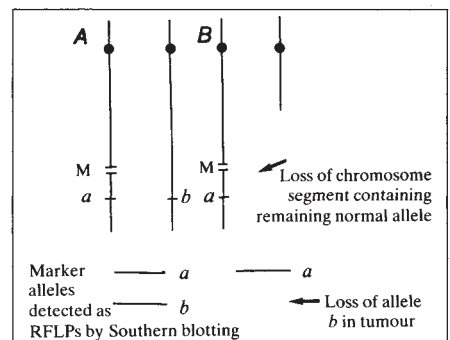
alleles at a single locus, mapped to the long arm of chromosome 13 (13q14), and individuals with the hereditary form of retinoblastoma have been shown to have inherited the loss of one copy of the gene in the germ line⁴. The retinoblastoma gene is regarded as the prototype of a class of tumour-suppressor genes whose presence in normal cells is required to prevent the emergence of a tumour, and which seem likely to have important functions in the regulation of normal cell proliferation and development. It is probable that the retinoblastoma gene and its product have both been identified. Excitement now is centred on what the gene does (which I will not discuss here) and on the possibility that these results, and the techniques which produced them, can be extended to the search for suppressor genes in other inherited cancers and in the common non-familial cancers.

Searching for suppressors

The germ-line loss of the first copy of the gene in familial retinoblastoma is visible at the cytogenetic level in a few cases. More often, there is no cytogenetic abnormality, and the inherited mutation is either a small deletion or, presumably, a point mutation. The loss of the second copy in a tumour cell, by contrast, often involves loss of a large piece, or all, of the chromosome⁵. This is of key importance because it suggests that in the search for suppressor gene losses, regions of chromosome loss in

the cancer cells will present a relatively large target — larger than that associated with the mutation in the germ line. Cytogenetic study of solid tumours is difficult, but with the increasing availability of DNA sequence polymorphisms which can be used as indicators (see figure), the search for chromosome losses has become relatively easy.

This approach has already led to the mapping of the loci of inherited predisposition for three of the inherited cancer syndromes: multiple endocrine neoplasia (MEN 1) to chromosome 11q; Von Hippel-Lindau syndrome (VHL; retinal angiomas, cerebellar haemangioblastoma and renal carcinoma) to 3p; and neurofibromatosis type 2 (NF-2; schwannomas of the VIII cranial nerve and meningiomas) to chromosome 22 (see Table 2). Another inherited cancer, familial polyposis of the colon, has also been mapped to 5q by the finding of an inherited chromosomal deletion. In each case, allele losses at the corresponding chromosomal locations have been found in the tumours. The double allele loss mechanism is therefore probably shared by most of the inherited cancers for which the loci have been identified, but may not turn out to be universal. Indeed, in multiple endocrine neoplasia type 2 (MEN 2), the predisposing gene for which has been mapped by genetic linkage alone to chromosome 10, allele losses at the corresponding locus in tumours have not yet been found.



Allele loss in tumours. A pair of chromosomes is shown in normal tissue (A) and in a tumour (B) from the same individual. The example shown is of an individual who has inherited a gene-loss mutation (M) which predisposes to cancer: loss of the remaining normal copy of the gene by somatic mutation leads to formation of the tumour. In other cases there may be no inherited predisposition, and loss of one or both copies of the gene occurs only within the tumour cells, as an event in tumour progression. *a, b*, Alleles at an arbitrary marker locus some distance from the inherited mutation, defined by restriction fragment length polymorphisms. The somatic mutational event is revealed by loss of one marker allele (here, allele *b*) in the tumour compared with normal tissue of the same individual. Where a predisposing germ-line mutation is present, the allele lost through the second, somatic, mutation is the one inherited from the unaffected parent.

Table 1 Examples of genetic change which contribute to the development of cancer

	Somatic cells	Germ line
Altered gene product	mutated oncogenes, for example, <i>ras</i> , <i>neu</i>	?metabolic polymorphism ?others not yet described
Altered gene expression	oncogenes, for example <i>myc</i> , <i>erb-B</i>	
Increase	'tumour suppressor genes' losses detected:	Inherited cancer syndromes, for example, retinoblastoma
Decrease or loss	cytogenetically — 'deletions' by DNA polymorphism — 'allele loss'	
		Syndromes of defective DNA repair: for example, Xeroderma pigmentosum, Bloom's.

Allele loss in common cancers

The allele losses which gave the clues to the location of the inherited genes for VHL and NF-2 are present in sporadically occurring tumours of the same histological type; and the same is true of the other inherited cancers which show a gene loss mechanism of predisposition, as the 'two-hit' model would predict. Can we therefore expect that, for example, the allele losses that commonly occur on chromosomes 11p and 13q in sporadic breast cancers, on chromosomes 17 and 18 in colonic cancers¹, or on 3p in lung cancers, reflect the existence of germ-line alleles that may contribute to inherited predisposition? At present, no one can be sure.

For breast cancer and for non-polyposis colonic cancer, the answer will soon come from genetic linkage studies in cancer families, using as markers DNA polymorphisms which map to these chromosomal regions. If positive, these linkage studies will also prove that the observed familial clustering⁵ is genetic in origin. For lung cancer, where familial cases are not so easily available, a more direct approach will be to search in the affected individuals for germ-line losses which correspond to those seen in the tumours — but as the figure shows, this will require DNA probes for sequences very close to the gene involved.

It is already clear that not all allele losses observed in tumours have a counterpart in the germ line. For example, in MEN 2, where the predisposing gene is mapped to chromosome 10, one-third of tumours show allele losses on chromosome 1p. If these losses reflect a second predisposing locus on chromosome 1, they should involve the wild-type chromosome inherited from the unaffected parent (see figure); but in one case of MEN 2, the allele loss in fact involves the chromosome inherited from the affected parent⁶. Of the multiple allele losses reported in colonic cancer (ref. 1; Table 2), it seems likely that some will correspond to loci of inherited predisposition, but that others reflect purely somatic events in tumour progression.

Dominance and dosage

There has been much confusion over which of the genetic changes in Table 1

should be called dominant and which recessive. Mutations giving rise to activated oncogenes are regarded as dominant because transfer of a mutated gene in transfection experiments is sufficient to induce an altered phenotype in the recipient cell. By contrast, in retinoblastoma and in the possibly analogous homozygous gene-loss mutations leading to tumorigenesis in *Drosophila*, there appears to be no detectable phenotypic effect of loss of the first copy of the gene. Moreover, in *Drosophila*, introduction of a single copy of the cloned wild-type allele into a mutant larva prevents tumorigenesis and reverses the effects of the mutation on cell differentiation⁷. These results are consistent with a truly recessive loss-of-function mutation in a gene which acts as a tumour suppressor. (The seeming

paradox that retinoblastoma behaves as a dominantly inherited cancer at the level of the individual is explained because loss of the single remaining wild-type allele is almost certain to occur in at least one retinal cell. As a result, almost all individuals will express the disease even though they inherit only a single copy of the mutant gene.)

The reality is less clear-cut than these examples suggest. In the case of the *Ha-ras* oncogene, there is evidence that cellular phenotype is determined by the relative number of copies in the cell of mutant and wild-type alleles; and similar dosage effects are found in experiments involving hybrids between normal and malignant cells⁸. In some of the inherited cancer syndromes, phenotypic effects could result from the loss or inactivation of a single copy of the putative 'suppressor' gene. The generalized proliferative disturbance in the colonic epithelium in colonic polyposis is presumably of this type: if a somatic mutation were required in addition to the germ-line loss, the expected result would be focal areas of abnormality. In fact there is no evidence for this or for loss of the wild-type allele at the polyposis locus until the stage at which malignancy occurs. So not all 'dominant' activated oncogenes are completely dominant, and not all loss of function mutations in tumour-suppressor genes

Table 2 Examples of allele losses in human tumours defined by DNA studies

Tumour	Site of allele loss in tumour	Corresponding loci associated with inherited predisposition
Retinoblastoma ¹²	13q	13q D, L
Osteosarcoma ¹²	13q	
Wilms ^{12,13}	11p	?11p D Note: not yet confirmed by linkage
Rhabdomyosarcoma ¹²	11p	
Hepatoblastoma ¹²	11p	
Hepatocellular carcinoma ¹⁴	11p	
Bladder ¹²	11p	
Renal carcinoma ¹²	3p	Family with t(3,8) ¹⁵ Von Hippel-Lindau 3p, L ¹⁶
Lung small cell ¹⁷⁻²¹	3p, 13q, 17p	
Other types ¹⁸⁻²⁰	3p (13q, 17p less frequent)	
Breast ^{12,22,23}	11p, 13q	
Stomach ²⁴	13q	
Colon ^{12,25-27}	5q, 17p, 18q, 22, others	Polyposis 5q D, L ²⁸
Insulinoma ¹²	11	MEN 1 11q L ¹²
Phaeochromocytoma ^{29,30}	1p, 22	MEN 2 10 L ^{31,32}
Medullary thyroid carcinoma ²⁹	1p	NF-1 17 L ^{33,34}
Meningioma ^{12,35}	22	MEN 2 10 L ^{31,32}
Acoustic neuroma ¹²	22	NF-2 22 L ³⁶
Melanoma ³⁷	Various	

p, Short arm; q, long arm; D, mapped by constitutional chromosome deletion; L, by genetic linkage. The aneuploidy of many cancer cells will result in considerable background 'noise' of allele losses on many chromosomes. The significance of an observed frequency of losses on any given chromosome is usually assessed by comparison with the frequency of losses on other chromosomes in the same set of tumours. In most of the reports cited here, extensive data of this sort are not available; and the decision that a particular loss should be included is therefore somewhat arbitrary. Several of the papers describe losses at lower frequencies on chromosomes other than those shown in the table, which may or may not be significant. In some cases, the likely significance of an observed loss is enhanced either by evidence of homozygous loss or by the identification, by genetic linkage, of a corresponding locus which confers inherited predisposition. Note that there is likely to be a strong bias in the chromosomes which are reported as showing losses, because some have been much more extensively tested than others. The table does not include data on chromosome losses from cytogenetic studies. (Ref. 12: original data cited in review.)

are truly recessive. Dosage of mutant alleles must be considered; and whether, in the case of mutations which occur exclusively in somatic cells, conversion from loss of one copy to loss of both copies is important in tumour progression.

Perhaps the phenotypic effects of the germ-line loss of a single copy of some suppressor genes could have been predicted from the evidence of the various syndromes of developmental abnormality in man which are associated with chromosome deletions. Overlap between these and the inherited cancer syndromes may be provided by syndromes of developmental abnormality in which tumours are an occasional feature. In dominantly inherited diaphyseal aclasia (multiple exostoses), for example, there is defective modelling of the ends of long bones with persistence of excessive cartilage and, in about 2 per cent of individuals, development of chondrosarcoma. In such cases, investigation of the tumours for allele loss might reveal the location of genes important in development.

A different speculation arising from gene dosage is prompted by reports⁹ that allele losses on chromosome 11p in a few cases of sporadic Wilms tumour show a bias towards retention of the paternally derived chromosome in the tumour. If these reports are confirmed by analysis of further cases, several explanations can be envisaged; but the implication is that there is a difference between the maternal and paternal chromosomes in this region, raising the possibility of epigenetic effects caused by genomic imprinting. There is no evidence for widespread functional hemizyosity in adult tissues as a result of inactivation of genes by this mechanism, but perhaps more subtle variations in expression could occur and could be revealed by bias in allele loss of this sort.

Tissue specificity

A remarkable feature of inherited cancer syndromes is their tissue specificity. Often, however, the pattern of tumours does not accord with any current ideas of physiology or cell lineage. The concurrence of retinoblastoma and osteosarcoma, or of colonic carcinoma, hepatoblastoma, thyroid cancer, carcinoma of the Ampulla of Vater and fibroblastic tumours in Gardner's syndrome, for example, is quite unexplained. Presumably, this implies either the existence of physiological or lineage relationships of which we are unaware, or that the same gene is used for a controlling function in several separate lineages. The allele losses in Table 2 suggest some possible patterns: for example, losses of chromosome 1p or 22 in tumours of neuroectodermal tissue, and of 3p in each of the different histological varieties of lung cancer; but others do not fit. But care is needed. The results are biased towards losses of alleles on a

few chromosomes because these are the ones which have been tested; moreover, most of the losses have not been precisely localized, and an association may easily be created or obscured by assuming that all losses on a single chromosome arm reflect changes at a single locus. More complete information is needed.

Curiously, the one case in which there is detailed information has merely added confusion. The retinoblastoma gene is expressed in a wide variety of tissues, yet germ-line mutation results in a clear predisposition only to retinoblastoma, osteosarcoma and possibly melanoma. The finding of structural changes or homozygous losses confined to the putative retinoblastoma locus in a few small-cell lung cancers¹⁰ and breast cancers¹¹ provides a further paradox. Without evidence of germ-line losses in these cases, it is likely that the double loss was a purely somatic event: but even so, some relationship between inherited predisposition to retinoblastoma and to these tumours might be expected. At present the only evidence of this is a reported increase in pre-menopausal breast cancer in the mothers of children with soft-tissue sarcoma: longer follow-up of retinoblastoma survivors is needed to be sure on this point. It seems we must distinguish between the tissue specificity of expression of the retinoblastoma gene and the expression of the phenotypic effect of its mutations. About the expression of the mutations, there is clearly much to learn. □

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Daedalus

With bated breath

NOISE is the curse of our civilization. From transport, industry and so-called entertainment it assaults us constantly. Daedalus is now fighting back. His new sound-deadening scheme exploits the fact that sound is strongly muffled by fog.

The reason is obvious. At the peaks of the sound-pressure cycle, water vapour condenses on the droplets, lowering the air-pressure, while at the troughs it evaporates from them, raising the pressure. The suspended droplets thus act as a buffer, absorbing the sound. A boiling kettle, however, is a limited defence against sonic assault. Its swirling mist does a better job of obscuring vision. This double inefficiency could be neatly reversed by making its mist droplets smaller. They would then cease to scatter light, being less than a wavelength across; and their total surface area would rise, letting them equilibrate faster and more perfectly with the water vapour in the air, and deadening its vibrations more completely.

But how to generate and stabilize ultra-small water droplets? The high surface tension of water gives their sharply curved surface an excessive vapour pressure; they evaporate, and their vapour goes to swell bigger droplets. A suitable detergent, however, could lower their surface tension and stabilize them against this form of evaporation. Many organic compounds are volatile in steam, and Daedalus is confident of finding a suitable detergent among them. Thus will be born the DREADCO Hushkettle®, which pours out a long-lasting, invisible fog of ultra-small water droplets. Their enormous total surface area will simply swallow up all sound entering the region.

At last the sonic menace will be beaten! The householder beneath the airport flight path; the passenger on public transport cringing from the cacophony of competing cassette-players; the restaurant patron shouting to converse above the alleged 'background' music — all will have a remedy close to hand. The soothing output of the Hushkettle will spread peace and quiet across the whole scene. Sound will still be audible a short distance from its source: owners of cassette-players will still be able to enjoy them, and friends will still be able to exchange close-whispered confidences. But such sounds will not get far. The throbbing public air will in effect be reduced to a set of private cells with no connection between them.

The Hushkettle could be misused, of course. It could reduce concerts and public meetings to total farce, and sabotage the courtship of cats, frogs and crickets. But its overall impact should be overwhelmingly positive.

David Jones

Canine distemper virus in seals

SIR—During the past four months more than 12,000 harbour seals (*Phoca vitulina*) have died in the seas of north-west Europe from a highly contagious disease, of which the clinical symptoms are similar to distemper in dogs¹. We initially isolated a herpes-virus and a picorna-like virus from organs of affected animals² but have since concluded that canine distemper virus (CDV), or a closely related morbillivirus, was the primary cause of the outbreak³. We now report the isolation of the virus using *in vitro* procedures, either directly or after a passage in dogs, its partial characterization and identification as CDV, and sero-epizootiological data that suggest that CDV probably does not commonly infect pinniped species.

Two six-week-old specified pathogen-free (SPF) beagle dogs (from a closed caesarean-derived breeding colony, which by regular serological screening was shown to be free of the viruses that are known to infect dogs) were each inoculated by the oculo-nasal and peritoneal routes with 2 ml of a 10 per cent (v/v) suspension of a pool of spleen, lung and intestinal lymph-node cells of three harbour seals, which had died from the disease in the Waddensea area. Both dogs developed mild clinical signs suggestive of CDV infection (Fig. 1) during the 12-day observation period, including respiratory symptoms from day 8 onward with a purulent nasal discharge. The body temperature of dog I rose above 39 °C on days 4–6 and in both dogs there was a lymphopaenia ($<1.5 \times 10^6$ lymphocytes per ml) on days 4–6, followed by an increase of circulating lymphocytes (up to 15×10^6 per ml) in the following five days. After co-cultivating peripheral blood mononuclear cells, collected 4, 5, 6 and 7 days after inoculation, with primary cultures of SPF dog-lung macrophages⁴, the presence of CDV or a related virus was shown by the formation of syncytia and confirmed by indirect immunofluorescence (Figs 1, 2a).

The presence of CDV or a related virus was further confirmed by negative con-

trast electron microscopy; nucleocapsids, with a length of approximately 1,400 nm and a diameter of 16.5–18.5 nm — characteristic of a paramyxovirus — were present in lysates of infected macrophages (Fig. 2b). After passage of the virus with cell-free culture medium to other primary SPF dog-lung macrophage cultures; syncytia and viral antigen were also demonstrated. CDV was also identified by the

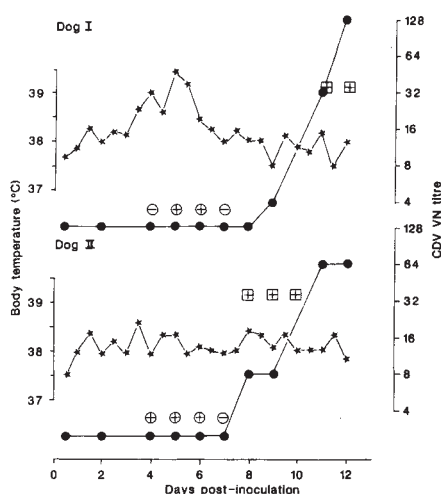


Fig. 1 Body temperature (x), presence of purulent nasal discharge (⊞), development of CDV-neutralizing antibodies (●) and virus isolation (⊕) during 12 days after inoculation of dogs I and II.

same criteria in monolayer cultures of African green monkey kidney cells (Vero cell-line) that had been inoculated with plasma samples from dog II at days 5 and 6. Plasma samples were also tested for the presence of CDV neutralizing antibodies^{3,4}. Both dogs developed antibodies within 10 days, with titres on day 12 of 64 and 12 per cent, respectively (Fig. 1).

These data show that CDV had been present in the suspension of pooled seal cells, used for inoculation and that the virus isolated was a strain of CDV with limited pathogenicity for SPF dogs. The fact we could propagate the virus in Vero

cells also indicates that it is of limited pathogenicity for dogs⁵, but it should be noted that even CDV strains that are generally highly pathogenic in dogs may cause only mild symptoms in SPF dogs^{4,5}.

During the observation period, none of the dogs developed antibodies to *Phocid herpesvirus-1* or the picorna-like virus², as judged by testing plasma samples in virus-neutralization assays. This indicates that neither of these viruses is responsible for the clinical signs in the dogs.

CDV, or a related virus, was also directly isolated from tissues of a harbour seal that had died in the Baltic Sea during the outbreak (kindly supplied by A. Bergman and T. Härkönen, University of Agriculture, Uppsala). Five days after inoculating monolayer cultures of canine kidney cells (MDCK-cell line) with a 10 per cent (v/v) suspension of spleen and intestinal lymph-node cells, the viral nucleoprotein was demonstrated by an indirect immunofluorescence test⁴ with a monoclonal antibody (designated A1146, supplied by C. Orvell, Karolinska Institute). Specificity of this immunofluorescence was confirmed by blocking with a hyperimmune anti-CDV SPF dog serum and not with a pre-immune serum of the same dog. Further passages of the virus in MDCK cells are being made.

To further investigate where and when the CDV infection had been introduced into seal populations, we extended our previous serological studies³ to samples collected between 1984 and 1988, before the outbreak has started. No CDV neutralizing antibodies (titre > 10) were detected in 134 harbour seals in the North sea area (courtesy E.J. Vedder, Seal Orphanage, Pieterburen), 60 ringed seals (*Pusa hispida*), 8 spotted seals (*Ph. largha*), 5 bearded seals (*Erignathus barbatus*), 12 sea lions (*Eumetopias jubatus*), 4 harbour seals (*Ph. vitulina*), 4 ribbon seals (*Histiophoca fasciata*) and 158 walrus (*Odobenus rosmarus*) in the West and East Behring Seas, and from 19 weddell seals (*Leptonychotes weddelli*) in the Antarctic (courtesy P. Reynders, National Institute for Nature Manage-

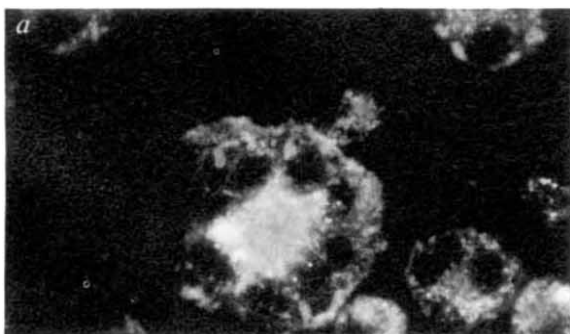


Fig. 2 a, Indirect immunofluorescence with serum of SPF dog after experimental CDV infection and fluorescein isothiocyanate-labelled anti-dog IgG conjugate, on SPF dog macrophage culture in which syncytia had developed four days after inoculation with seal cell suspension. b, Negative contrast (PTA) electron micrograph of full-length nucleocapsid taken from macrophage culture lysate. Scale bar, 100 nm (courtesy J.S. Teppema, National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands).

ment, Texel).

As the humoral response after natural CDV infection persists for several years, and perhaps even for life, in the dogs, and assuming that this also holds true for pinnipeds we might conclude that CDV does not commonly infect pinniped species. Because acquired immunity to this virus is apparently not present in these animals, there is a potential risk that seals in the Canadian and American seas may also be subject to CDV infection. Further sero-epizootiological studies among pinniped species at different locations, and biological and biochemical comparison of virus isolates from terrestrial and aquatic mammals may elucidate the origin of the outbreak of CDV infection in seals.

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Confirmation of cause of recent seal deaths

SIR—Osterhaus and Vedder recently presented serological evidence associating canine distemper virus (CDV) or a closely related morbillivirus with the current high mortality in European seals (*Nature* **335**, 30; 1988). Here, we confirm such a virus as the cause of the deaths and report the isolation of a morbillivirus from an affected seal.

We have necropsied 17 dead or seriously ill seals (*Phoca vitulina*) found on the coast of Northern Ireland since 3 August 1988. Our main necropsy findings were severe pneumonia, encephalitis and ophthalmitis. Histopathological brain lesions were typical of a viral encephalitis

and we saw many intracytoplasmic and intranuclear acidophilic inclusion bodies (*a* in the figure) characteristic of CDV infection in neurons and astrocytes (Dugworth, D.L. in *Pathology of Domestic Animals* (eds Jubb, J. *et al.*) Academic, London; 1985). Pulmonary lesions were characterized by diffuse interstitial pneumonia. There was proliferation of type II pneumonocytes resulting in syncytia formation in the lungs of several seals. Although such changes are consistent with CDV infection, we did not see inclusion bodies in the lungs of any of the seals.

We examined frozen sections of pneumonic lung from six seals (*P. vitulina*) by indirect immunofluorescence using a polyclonal hyperimmune antiserum raised in rabbits to the Onderstepoort strain of CDV. Coverslip preparations of cell cultures infected with this strain were used as positive controls. We detected positive immunofluorescence in the lungs of three of the six seals. Immunofluorescence had mainly a diffuse cytoplasmic distribution pattern but large intracytoplasmic inclusion bodies also stained.

We also isolated a virus by culture of cells from the kidney of a seal that had encephalitis and ophthalmitis. The virus produces cell vacuolation and syncytia, characteristic effects of a morbillivirus. Positive immunofluorescence using our hyperimmune antiserum confirms the cultured virus as a morbillivirus (*b* in the figure).

Our finding of histopathological lesions and inclusion bodies similar to those of CDV infection in other species, and demonstration of morbillivirus antigen in diseased lung and isolation of a morbillivirus from an affected seal provide very strong evidence for an aetiological role of CDV or a closely related morbillivirus as a primary cause of seal mortality. Further characterization of the virus is in progress.

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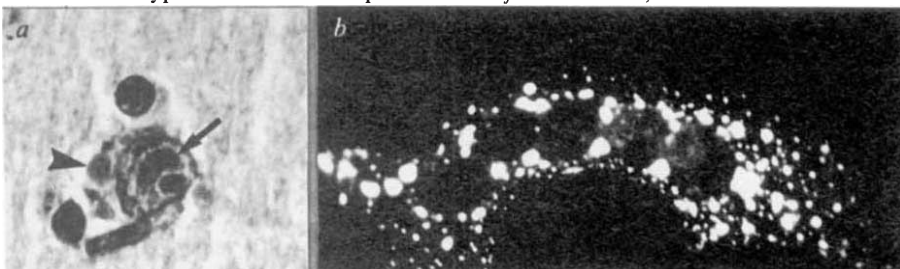
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a, Intranuclear (arrow) and intracytoplasmic (arrowhead) inclusion bodies in neuron of seal with encephalitis. *b*, Morbillivirus antigen in syncytium in seal kidney cell culture.

Ancient carbon sources of atmospheric methane

SIR—Sackett and Barber¹ comment on the observation² that 32 per cent of atmospheric methane is ¹³C-free and therefore of an ancient or primordial origin. They quote quantitative aspects of the possible emission of unburnt methane to the atmosphere by the petroleum industry, and of the possible release of methane from fresh tar. They do not, however, give the quantities with which this would have to be compared to account for the observation.

The total mass of carbon in the form of atmospheric methane (at a volume proportion of 1.6 parts per million) is 3.37×10^{15} g, of which the 32 per cent that is not from locally recycled plant material accounts for 1.08×10^{15} g. Because atmospheric methane has an average lifetime (against oxidation) of 5–7 yr, 1.54 – 2.16×10^{14} g of this quantity will be replaced each year. This compares with the “several” units of 10^{12} g suggested by Sackett and Barber to come from venting of unburnt methane by the petroleum industry, and five such units from the exposure of fresh tar. The petroleum industry thus seems to be responsible for at most a few per cent of the atmospheric methane.

Addition of methane to the atmosphere has a far larger greenhouse effect³ than a similar quantity of carbon in the form of CO₂, as the CO₂ infrared bands are already practically saturated. The natural seepage of methane from gas fields provides a large contribution, which is reduced, demonstrably in some cases, by the production of gas from those fields. Because the gas produced in this way is generally burnt to CO₂ (and water) it will contribute much less to the greenhouse effect than if it were allowed to seep into the atmosphere as methane. Therefore, it is possible that the gas-producing industry (although not the oil industry) may in fact be helping to restrain the greenhouse effect.

It may also be interesting to relate the quantity of ¹³C-free carbon now observed to the supply of juvenile carbon that appears to have entered the atmosphere over geological times and resulted in the deposition of carbon in the sediments, in carbonates and other forms. The total amount of carbon so laid down in the past 500 million years is generally judged to be of the order of 10^{25} g. An annual supply rate of 2.16×10^{14} g (derived above) would provide 1.3×10^{23} g in this time.

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Multifractal phenomena in physics and chemistry

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The neologism 'multifractal phenomena' describes the concept that different regions of an object have different fractal properties. Multifractal scaling provides a quantitative description of a broad range of heterogeneous phenomena.

A WIDE range of complex structures of interest to physicists and chemists have in recent years been quantitatively characterized using the idea of a fractal dimension; a dimension that corresponds in a unique fashion to the geometrical shape under study and that often is not an integer¹⁻⁷. The key to this progress has been the recognition that many objects with random structure possess a scale symmetry. Scale symmetry implies that objects look the same on many different scales of observation.

To be more specific, consider an object with fractal dimension d_f . Imagine that we digitize the object by representing it by the pixels of a computer. If a unit mass is associated with each pixel, the total mass M of the object corresponds to its volume and its 'density' $\rho \equiv M/L^d$ is a measure of the fraction of d -dimensional space occupied by the object. Here L is a characteristic diameter, such as the caliper diameter or radius of gyration. If, however, $d_f < d$, the mass increases more slowly than L^d as the size of the object increases; for example, if we double L ($L \rightarrow L' = 2L$) then M increases by a power less than

2^d (that is, $M \rightarrow M' = 2^{d_f} M < 2^d M$). Thus the density decreases ($\rho \rightarrow \rho' = 2^{d_f-d} \rho < \rho$).

As long as a unit mass is associated with each pixel, a single scaling exponent d_f characterizes the structure of the object. In recent years, however, very interesting phenomena have been studied which seem to require not one but an infinite number of exponents for their description. Such multifractal phenomena have recently become an extremely active area of investigation and here we provide the non-specialist with a brief introduction to them. There are many types of multifractal phenomena but we shall concentrate on two examples, the behaviour of complex surfaces and interfaces⁸, and fluid flow in porous media.

Complex surfaces

Figure 1a shows an object formed by a process called diffusion-limited aggregation (DLA)^{9,10}; such structures arise naturally in many processes currently of interest to physicists and chemists, ranging from electrochemical deposition^{11,12}, thin-film

Fig. 1 The harmonic measure for a 50,000 particle off-lattice two-dimensional DLA aggregate. *a*, The cluster; *b*, all 6,803 perimeter sites that have been contacted by at least one out of 10^6 random walkers, following off-lattice trajectories. *c*, All the perimeter sites that have been contacted 50 or more times. *d*, The sites that have been contacted 2,500 or more times. The maximum number of contacts for any perimeter site 8,197, so that $p_{\max} = 8.2 \times 10^{-3}$ (after ref. 27).

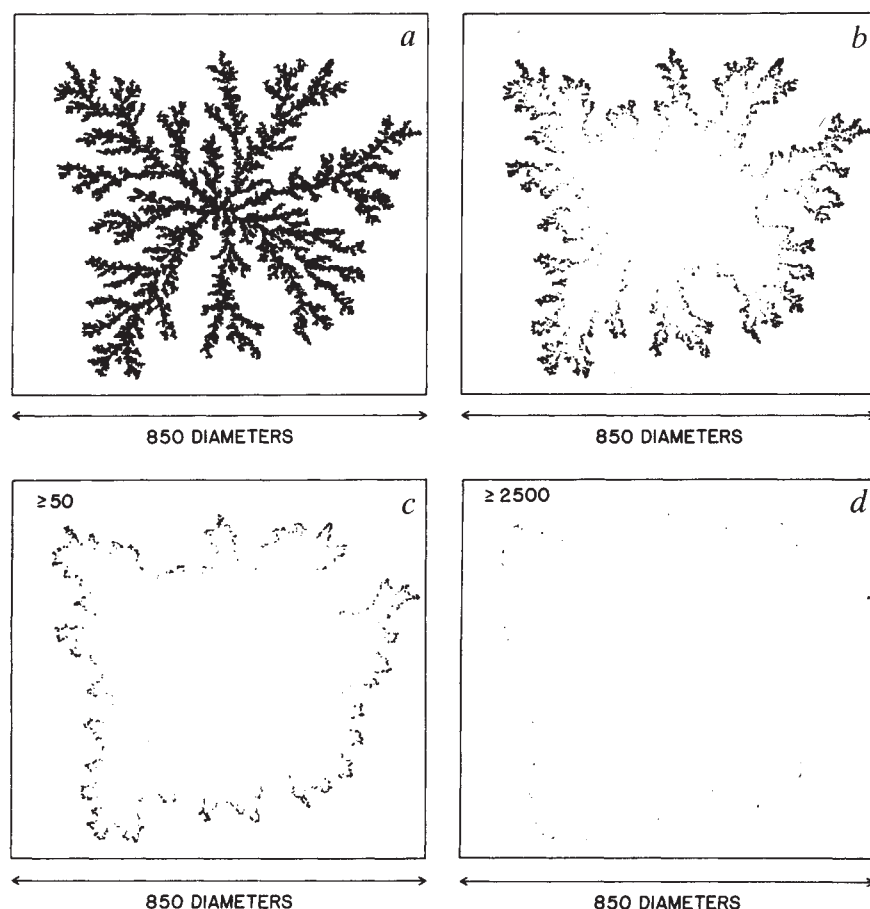


Fig. 2 Comparison between: *a*, *b*, the distribution functions $n(p)$ and *c*, *d*, the critical exponents $\tau(q) = (q-1)D(q)$ for viscous fingering patterns. *a*, Theoretical and experimental values for $n(p)$, where $n(p)\delta p$ is the number of perimeter sites with growth probabilities in the range $[p, p + \delta p]$. The simulated patterns and their growth probabilities were obtained³¹ using the dielectric breakdown model. The growth probabilities for the experimental patterns were obtained by numerically solving Laplace's equation in the vicinity of a digitized representation of the pattern with absorbing boundary conditions on the sites occupied by the pattern. Similar results were obtained for small α by directly subtracting two successive experimental patterns. *c*, Theoretical and *(d)* experimental values for $\tau(q)$ in both cases was obtained numerically as in *a* and *b* (refs 31 and 32).

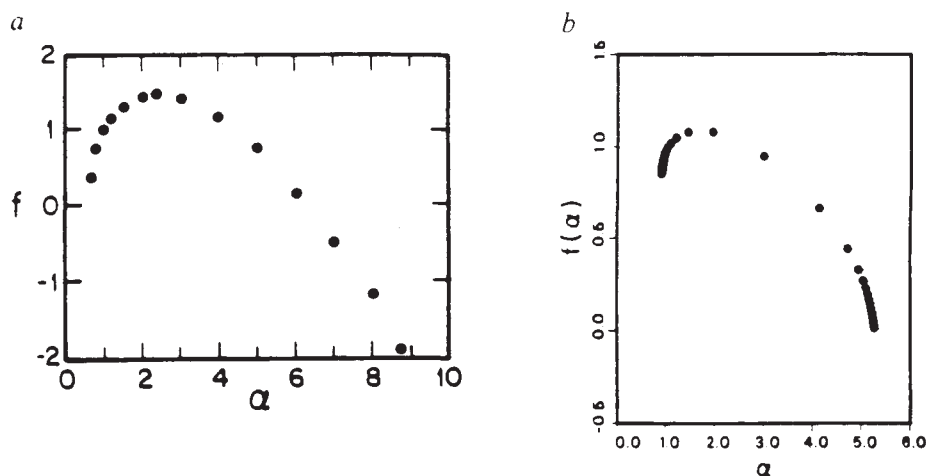
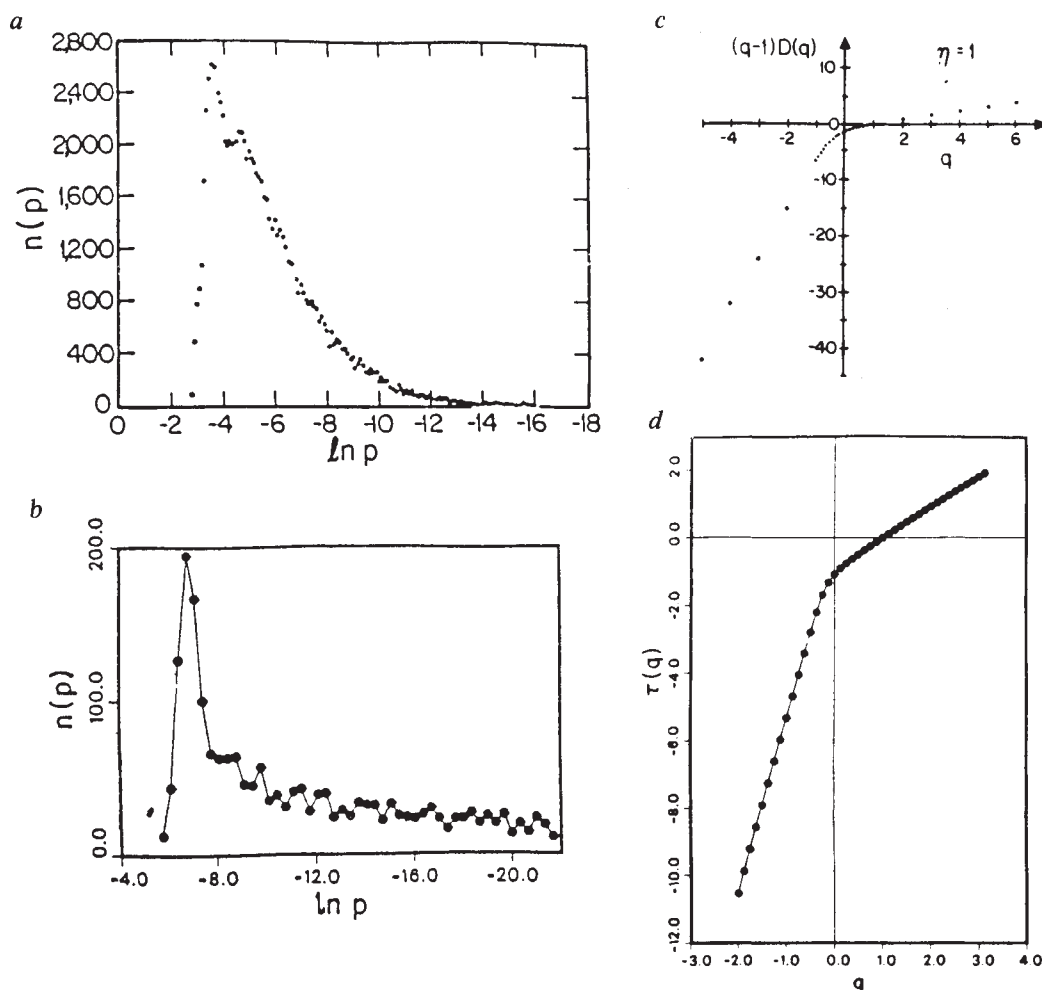


Fig. 3 Comparison between theoretical (*a*) and experimental (*b*) plots of the function $f(\alpha)$ (see Box 1) (refs 31 and 32).

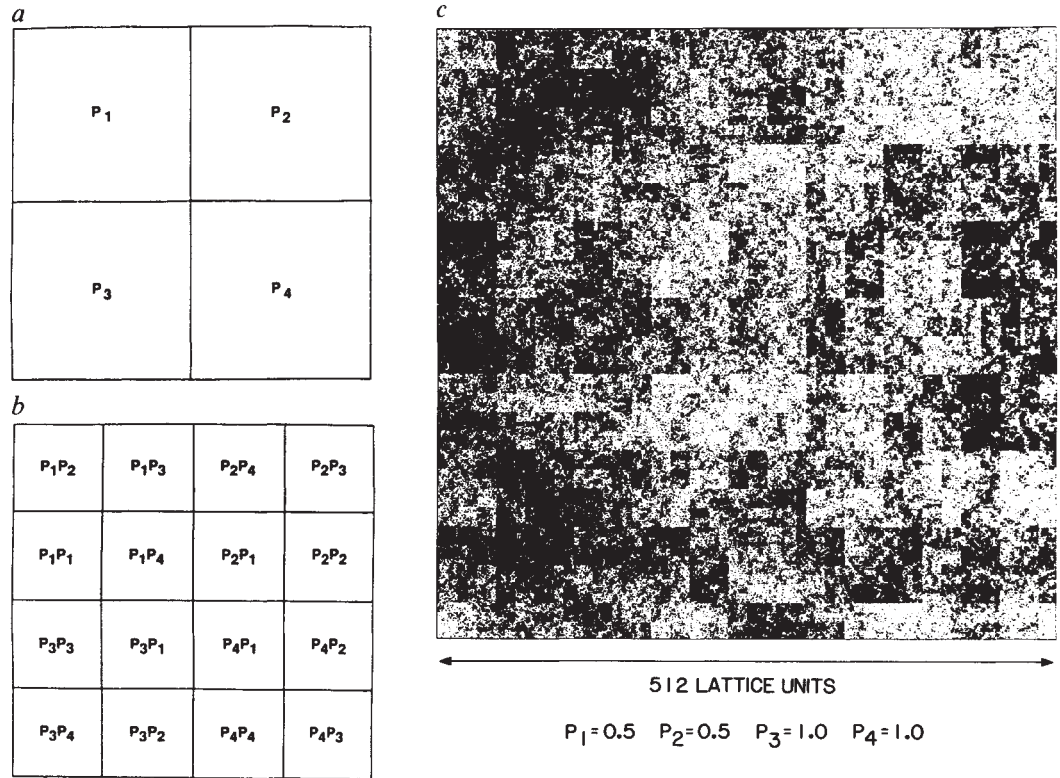
morphology¹³ and dendritic solidification¹⁴⁻¹⁷ to various 'breakdown phenomena' such as dielectric breakdown^{18,19} viscous fingering²⁰⁻²⁴, and chemical dissolution^{25,26}. If patterns such as the one shown in Fig. 1*a* are digitized, the fractal dimension $d_f \approx 1.7$ is obtained. Thus $d_f - d = -0.3$, and ρ decreases as the object grows due to the presence of 'fjords' whose size increases as the DLA cluster grows.

What is meant by the surface of the DLA cluster in Fig. 1*a* depends on the way it is to be measured. The exposed tips define one surface that is most likely to matter when diffusion is the essential feature, for example, if the surface is probed by parti-

cles undergoing random-walk motion. Figure 1*b-d* shows where the same object has been 'hit' by 10^6 random walkers²⁷, highlighting the surface sites touched by one, 50 and 2,500 random walkers respectively. Any of these three figures could be used to define the accessible surface, but the actual surfaces shown in Fig. 1*b-d* differ a great deal from one another. This example emphasizes that there is no unambiguous definition of the surface of this object.

An unambiguous quantity is the 'hit probability' p_i , defined as the probability that surface site i is the next to be hit. Operationally, we calculate $p_i \equiv N_i / N_T$, where N_i is the number

Fig. 4 A multifractal lattice: *a*, generator; *b*, second stage of construction; *c*, results after 8 generations. Lattice shown contains 2^8 pixels along each edge and shading is proportional to the value of π_i for pixel *i*. Here π_i is the product of the 8 probabilities assigned to that pixel as a result of the 8 generations (ref. 61).



of trajectories that hit site *i* and $N_T = \sum_i N_i$ is the total number of trajectories (for the example of Fig. 1 $N_T = 10^6$). The set of numbers p_i may be used to form a probability distribution function $n(p)$, where $n(p)\delta p$ is the number of p_i in the range $[p, p + \delta p]$, as shown in Fig. 2. This probability distribution, like all probability distributions, is characterized by its moments

$$Z(q) = \sum_p n(p) p^q \quad (1)$$

A central dogma of critical point phenomena has been the statement that the probability distributions that arise are characterized by only two independent exponents²⁸. This means that we obtain no more information about the system by studying increasingly higher moments: moment $q+1$ is described by an exponent related to that of moment q by a simple gap exponent Δ . In general, one finds that

$$Z(q) \sim L^{-\tau(q)} \quad (2)$$

If the central dogma were correct, then $\tau(q)$ would be a linear function of q so that only two independent exponents would be needed to specify $\tau(q)$. It was discovered recently that this idea fails for the probability distribution $n(p)$ for DLA: both simulation^{27,29-31}, (Fig. 2c) and experiments³² (Fig. 2d) show that $\tau(q)$ is a continuous curve. An infinite hierarchy of independent exponents is required. The Legendre transform $f(\alpha)$ of the function $\tau(q)$ contains the same information as $\tau(q)$ itself (see Fig. 3 and Box A), and is the characteristic customarily studied when dealing with multifractals^{27,30-42}.

Fluid flow in complex media

Consider a second example, fluid flow in random porous media. Such flow is customarily represented by considering an idealized network of bonds, a fraction p of which are open, and the remaining fraction $1-p$ of which are blocked⁴³. For p lower than a critical value p_c , termed the percolation threshold, no fluid passes across a macroscopic system. At p_c a single macroscopic cluster appears, called the incipient infinite cluster, which carries fluid across the entire system. The singly connected bonds

A. Analogies of multifractals with thermodynamics and multifractal scaling

Consider the sum in equation (1) in the form

$$Z(q) = \sum_p e^{F(p)} \quad (A1)$$

where

$$F(p) = \log n(p) + q \log p \quad (A2)$$

The sum in (A1) is dominated by some value $p = p^*$, where p^* is the value of p that maximizes $F(p)$. Thus

$$Z(q) \sim e^{F(p^*)} = n(p^*) (p^*)^q \quad (A3)$$

For fixed q , p^* and $n(p^*)$ both depend on the system size L , leading one to define the new q -dependent exponents α and f by

$$p^* \sim L^{-\alpha}; \quad n(p^*) \sim L^f \quad (A4)$$

Substituting (A4) into (A3) gives

$$Z(q) \sim L^{f - \alpha q} \quad (A5)$$

Comparing (A5) with equation (2), we find the desired result

$$\tau(q) = q\alpha(q) - f(q). \quad (A6)$$

From (A2) it follows that

$$\frac{d}{dq} \tau(q) = \alpha(q). \quad (A7)$$

Hence we can interpret $f(\alpha)$ as the negative of the Legendre transform of the function $\tau(q)$

$$f(\alpha) = -(\tau(q) - q\alpha) \quad (A8)$$

where $\alpha = d\tau/dq$. The function $Z(q)$ is formally analogous to the partition function $Z(\beta)$ in thermodynamics, so that $\tau(\beta)$ is like the free energy. The Legendre transform $f(\alpha)$ is thus the analogue of the entropy, with α being the analogue of the energy E . Indeed, the characteristic shape of plots of $f(\alpha)$ against α (compare Fig. 3) are reminiscent of plots of the dependence on E of the entropy for a thermodynamic system.

B. Random multiplicative processes

[A cautionary note for random sampling algorithms]

Multifractal phenomena seem to be associated with systems where the underlying physics is governed by a random multiplicative process. A simple random additive process might be the sum of 8 numbers, each number being chosen to be either $a-1$ or $a+1$ (this has a geometrical interpretation as an 8-step random walk on a one-dimensional lattice). Similarly, a simple random multiplicative process could be the product of 8 numbers, each number randomly chosen to be either $1/2$ or $a/2$ (S. Redner, personal communication). The results of simulations of such a process are shown in Fig. 5. The y -axis is the running average of the product after R realizations and the x -axis is the number of realizations R .

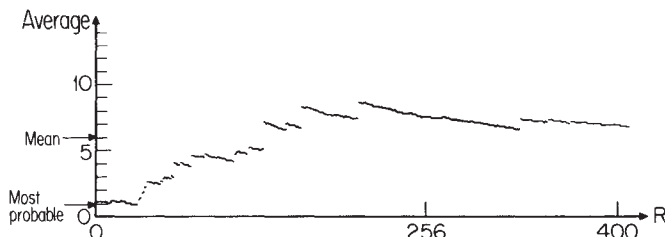


Fig. 5 A computer simulation of a random multiplicative process in which a string of 8 numbers is multiplied together. Each number is chosen with equal probability to be either 2 or $1/2$. The limiting or asymptotic value of the product is $(5/4)^8 = 5.96$. The simulations do not give this value unless the number of realizations R is approximately the same as the total number of configurations of this product, $2^8 = 256$. Simulation provided by R. Selinger.

In total there are 2^8 , or 256, possible configurations of such random products. Normally, random sampling procedures give approximately correct answers when only a small fraction of the possible 256 configurations has been realized. Here, however, one sees from Fig. 5 that the correct asymptotic value of the product is attained only after ~ 256 realizations (S. Redner, personal communication). Monte Carlo sampling of only a small fraction of the 256 configurations is doomed to failure because a rare few configurations—consisting, of, say, all 2s or seven 2s and a single $1/2$ —bias the average significantly and give rise to the upward steps in the running average shown in Fig. 5.

A simple random multiplicative process that gives rise to multifractal phenomena is found in the simple hierarchical model of the percolation backbone shown in Fig. 6. If the potential

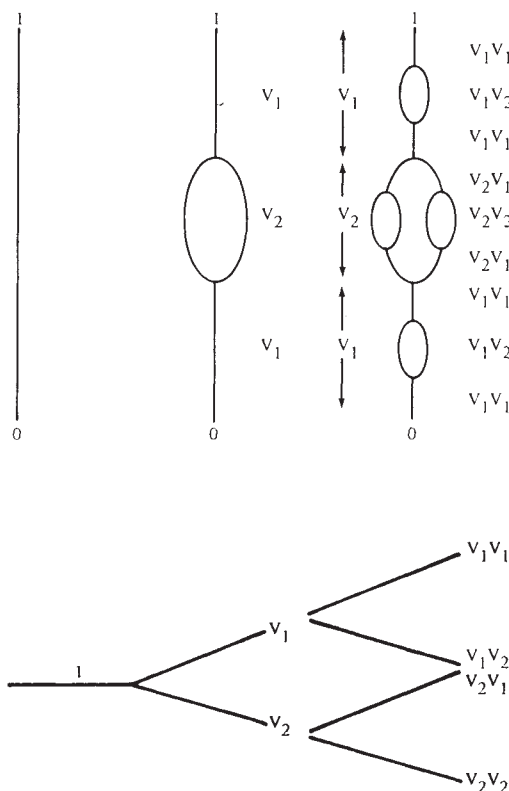


Fig. 6 A hierarchical model of the percolation backbone which displays multifractal scaling of the potential distribution $n(V)$. A unit potential is applied across the extremities of the cluster. The potentials shown are given by $V_1 = 2/5$ and $V_2 = 1/5$ (after ref. 37).

drop across the singly connected links is V_1 and that across the multiply-connected links is V_2 , then when this structure is iterated, the potential drops across each of the bonds are products of the potential drops of the original structure. For this hierarchical structure $Z(q) = (V_1^q + V_2^q)^N$, where N is the number of iterations carried out. It turns out that $Z(q)$ obeys a power-law relation of the form of equation (2), with an infinite hierarchy of exponents given by $\tau(q) = 1 + \log[V_1^q + V_2^q]/\log 2$. To obtain this result, the relation $N_1 = L^{3/4}$ must be used, where N_1 is the number of singly connected bonds⁵⁴⁻⁵⁷.

of this incipient infinite cluster carry the entire current and so sustain the largest potential drops, whereas the multiply connected bonds partition the current among them and so have smaller potential drops. The analogue of the hit-probability distribution $n(p)$ for DLA is the potential-drop distribution $n(V)$, where $n(V)dV$ is the number of bonds whose current lies in the interval $[V, V+dV]$ ⁴⁴⁻⁵³. The distribution $n(V)$ is characterized by its moments and the analogue of (1) is $Z(q) = \sum n(V)V^q$. If the size of the random network is doubled, the distribution function $n(V)$ changes and so do the moments $Z(q)$. An infinite hierarchy of exponents $\tau(q)$ is found and again $\tau(q)$ is not linear in q .

It is natural to ask why the constant gap or single exponent idea breaks down when studying the surface of a DLA cluster. The key idea is that the underlying probability distribution $n(p)$ develops a long 'tail' extending to extremely small values of the variable p (Fig. 2). For DLA, this tail arises from the presence of extremely deep cluster 'fjords'. As the cluster grows, the hit probability p_i for all cluster sites decreases. The hit probability p_i for a site deep in a fjord, however, decreases much faster

than p_i for a site on the exposed tips. Thus the long tail of the $n(p)$ distribution shifts its relative weight to smaller and smaller values of p as the cluster grows.

Similarly for flow in porous media, the distribution function $n(V)$ has a long tail extending to extremely small values of the potential V . This tail arises from the presence of large multiply connected regions of the network, termed blobs⁵⁴⁻⁵⁷. When $L \rightarrow L' = 2L$, the characteristic size of the largest blobs increases from M to $M' = 2^{1.62}M$ (ref. 58). Thus the minimum potential drops decrease dramatically and the distribution function $n(V)$ has a larger fraction of its weight from very small values of V .

Generality

It is not necessary to have a fractal structure to find multifractal phenomena. For example, consider the electric field E_i at every point i on the surface of a charged needle—a non-fractal object of dimension one. The set $\{E_i\}$ of electric-field values is formally analogous to the set $\{p_i\}$ of DLA growth probabilities, and indeed one finds that the $\{E_i\}$ also form a multifractal set^{27,35}. A question that arises naturally concerns the conditions under

which multifractal phenomena can be expected. As the example of the needle suggests, it is necessary to define a 'measure' on an object such that this measure has a different fractal dimension in different regions of the object⁵⁹. Thus when the length of a needle is doubled, the electric field near its tip changes by a factor which differs from the factor by which the electric field near the centre changes. Similarly, when the mass of DLA is doubled, the growth probability near the tips of a DLA structure changes by a factor different from the growth probability deep in the fjords. This is because the screening in the deep fjords increases dramatically with increasing cluster size.

Multifractal theory permits the characterization of complex phenomena in a fully quantitative fashion. Just as completely random phenomena in nature may generate shapes that are fractal, phenomena with spatial correlations are sometimes multifractals. For example, randomly porous media are traditionally modelled by the random-resistor network of percolation theory: the resistance of each element corresponds to the permeability in a suitable digitization of the porous medium. Although this model captures much of the essential physics, it neglects the phenomenon of spatial correlation that in turn leads to both short- and long-range heterogeneities in the porous medium. For this reason, it has been recently proposed that atmospheric turbulence and porous media should be modelled by a multifractal lattice⁶⁰⁻⁶⁴. This is obtained by a random multiplicative process (see Fig. 4 and Box B). Transport in such a lattice can be anomalously slow, just as it is in the random-resistor network model. But the exponent d_w describing the anomaly can be continuously tuned; in fact the slowing down can, under suitable conditions, become large without limit. A similar model has been solved analytically in one dimension⁷⁶.

Analogous phenomena are found for a wide variety of systems; in fact, multifractal phenomena were first found in studies

of fluid turbulence^{65,66}, and in analysis of non-linear dynamical systems⁶⁷⁻⁶⁹. Recently it has been demonstrated³³ that experimental data concerning the onset of turbulence can be analysed using a method derived from multifractal theory. Various multifractal sets have been mapped on to the thermodynamics of one-dimensional spin models⁷⁰. In a study of the depletion of a diffusion substance near an absorbing polymer it has been found that the scaling with distance r of each moment of the Laplace field is governed by an independent exponent⁷¹.

Several authors have examined the multifractal properties of random walks⁷²⁻⁷⁴. In particular, it has been shown⁷²⁻⁷⁴ that the fractal dimension d_f is a member of a continuous set of scaling exponents; consideration of the entire hierarchy of scaling exponents provides a more complete description of random walks than was possible previously. The main idea is to characterize an infinite walk with an exponent α that measures how fast its total probability decays to zero with increasing mass of the walk. The analogue of the function $f(\alpha)$ discussed above is the growth rate $z(\alpha)$ for the subset of walks with decay rate α . The analogue of $\tau(q)$ is the Legendre transform of $z(\alpha)$. A log-normal distribution has been found for the first-passage time in percolation⁷⁷, and some understanding of the conditions under which such a log-normal distribution will occur has also developed recently^{37,75}.

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Patterns of the cosmic microwave background from evolving string networks

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A network of cosmic strings generated in the early Universe may still exist today. As the strings move across the sky, they produce, by gravitational lensing, a characteristic pattern of anisotropies in the temperature of the cosmic microwave background. The observed absence of such anisotropies places constraints on theories in which galaxy formation is seeded by strings, but it is anticipated that the next generation of experiments will detect them.

Presently the only known physical mechanisms for producing the observed cosmological inhomogeneities involve either cosmic strings or quantum fluctuations produced during an inflationary epoch¹. Cosmic strings are linear topological defects that are predicted by some grand unified theories to form during a spontaneous symmetry-breaking phase transition in the early Universe². In inflationary scenarios only the density inhomogeneities and a gravitational wave background survive to be detected during the present epoch, and neither of these would carry any unambiguous signature of their inflationary origin. In contrast, in the string scenarios the strings themselves survive to the present epoch and have the unique observable signature of producing discontinuous jumps in the intensity of the microwave background radiation (MBR)^{3,4}.

To estimate the sensitivity of anisotropy experiments to the temperature patterns expected from strings, one requires knowledge of the statistical properties of the string networks. It is expected that these networks evolve towards an equilibrium 'scaling solution', in which case the present properties of the network may be determined without knowledge of the initial conditions. Numerical simulations of string networks in an expanding universe have demonstrated the existence of the scaling solution and revealed some of the properties of this equilibrium⁵⁻⁷. We have taken one such matter era simulation and used the time evolution of the network to estimate the temperature pattern, viewed at a distance, that would be produced in the simulation by the strings.

Geometry of the simulation

For details of numerical technique and a description of the results relating to the strings see refs 5 and 6. The string simulation was done in a cube of fixed co-moving size with periodic boundary conditions. The initial horizon size ($3t$) was 0.25 times the cube size and the simulation continued for an expansion factor of 16, so that the final horizon size was equal to the cube size. If z_i is the epoch (redshift) at the start of the simulation then the co-moving size of the box is

$$L = \frac{2}{0.25} \frac{c}{H_0} (1+z_i)^{-1/2} = 759 \sqrt{\frac{1,000}{1+z_i}} h_{100}^{-1} \text{ Mpc} \quad (1)$$

where H_0 is the Hubble constant and $h_{100} = H_0/100 \text{ km s}^{-1} \text{ Mpc}^{-1}$. The temperature shift was calculated for three planes of photons moving in the x , y and z directions, each of which had time to cross 0.75 of the cube length during the run. The geometry is such that these are approximately the

photons we would see at a single instant if $z_f = z_i/16 \gg 1$. The temperature shifts we have calculated for these photons are only those from the $L \times L \times 0.75L$ subset of our cube which the photons have swept out. These photons will subtend a square on our sky with angular size

$$\theta_L = \frac{\Theta_{H_i}}{0.25};$$

$$\Theta_{H_i} = \frac{1}{\sqrt{z_i}} \text{ radians} = 1.8^\circ \sqrt{\frac{1,000}{1+z_i}} \quad (z_i \gg 1) \quad (2)$$

where Θ_{H_i} is the apparent angular size of the horizon at z_i . In the matter era the projected angular length of string in the redshift interval $[z_i, z_f]$ scales as

$$\theta_{\text{string}} \propto \sqrt{z_i} - \sqrt{z_f} \quad (z_f \gg 1) \quad (3)$$

for a random patch of sky (here we assume implicitly that the cosmic density parameter $\Omega_0 \approx 1$). Thus most of the visible strings are concentrated at high redshifts near the surface of last scattering. We take the simulation volume to be up against the surface of last scattering: $z_i = z_{ls}$. We are thus missing no visible strings from behind the simulation cube, but we are missing strings from between the cube and us. Equation (3) tells us that we have included only 75% of the string that should be included for $z_{ls} \approx 1,000$ (the correct value if the universe was not reionized soon after recombination). To compensate for this we have placed two simulation volumes end to end, thus covering an expansion factor of 256. The photons we are considering start traversing the second simulation volume when the value of $1+z$ is 16 times less than that when they started traversing the first simulation volume. Equation (2) then implies that the co-moving size of the second simulation volume, L' , must be four times larger than that of the first, L , so we need to consider only $\frac{1}{16}$ of the photons traversing the second volume. The composite map now includes all but 6% of the correct number of strings for $z_{ls} \approx 1,000$. On the other hand, if the universe becomes reionized at high redshifts then z_{ls} could be as low as 30, in which case we have included about 18% too many strings. We feel that ours is a good compromise, given uncertainties in reionization. Equation (2) shows that the redshift of last scattering also determines the angular size of the region for which we have calculated the temperature pattern. Thus the apparent angular size of our temperature pattern should be in the rather large range $\theta_L = 7-40^\circ$. Although observers might wish for firmer predictions, theoretical uncertainties simply do not yet allow them. The ionization history of the universe will depend on the

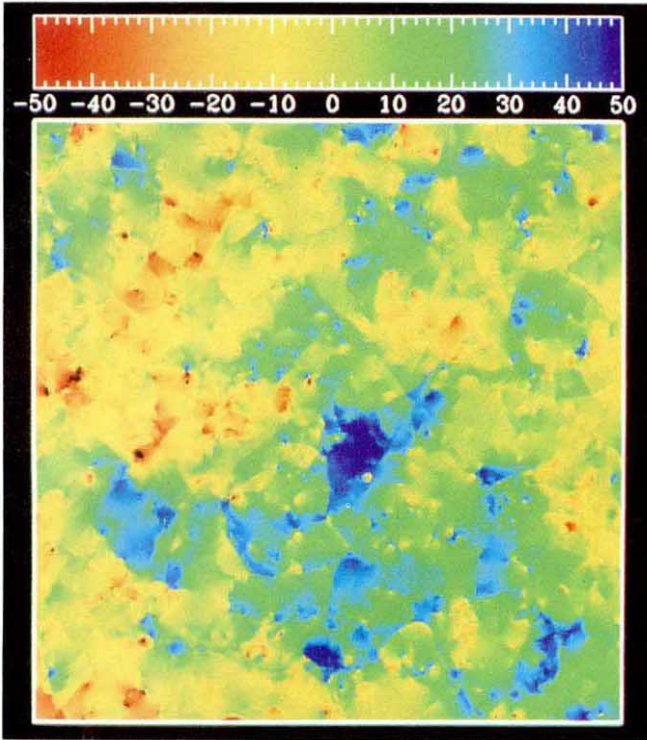


Fig. 1 The $\Delta T/T$ map calculated from a numerical simulation of cosmic string evolution. The map was constructed by combining two maps, each of which covered an expansion factor of 16. With the proper rescaling of the second map, the combined map covers an expansion factor of 256. The angular size of the map is $7.2^\circ \sqrt{1,000/(1+z_{ls})}$ where z_{ls} is the redshift of the surface of last scattering. The scale at the top gives $\Delta T/T$ in units of $G\mu/c^2$.

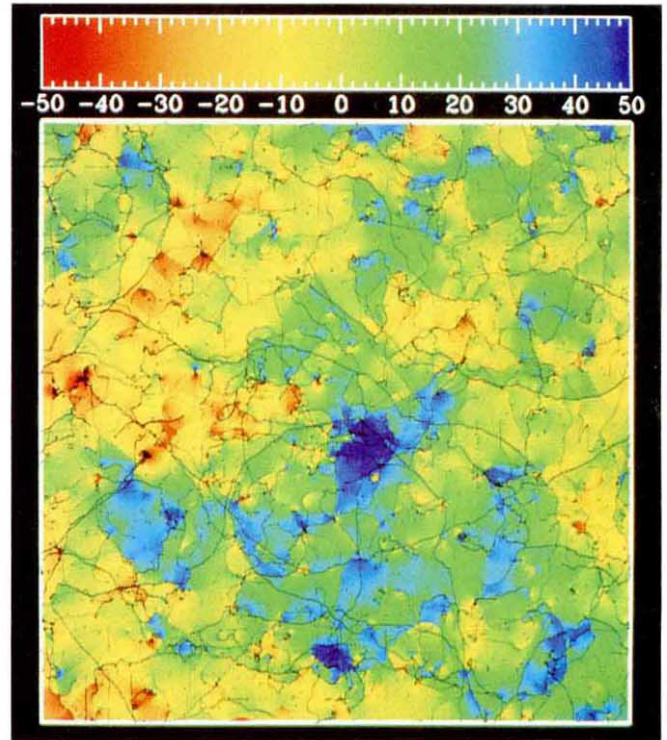


Fig. 2 Same as Fig. 1 but with projected positions of strings on the past light-cone of the observer superimposed in black. Some lengths of string appear as dotted curves rather than solid curves because of the discrete sampling of the string in the simulations.

efficiency of primordial star formation, which is not well understood.

The temperature calculation

Because of their relativistic motions^{3,4}, the gravitational fields of strings produce significant anisotropies in the microwave background. To calculate the temperature pattern from this effect we have used a Fourier version of the flat-space formalism given in ref. 8. With this method we can impose periodic boundary conditions on the temperature pattern consistent with the periodic boundary conditions of the string simulation. This formalism is not generally applicable to an expanding universe. However, as we shall describe, we expect the formalism to give a reasonable estimate of the anisotropy. A proper cosmological treatment of the anisotropy with present techniques would be much more computer-intensive and would thus yield results with a much smaller dynamic range. Furthermore, the peculiar stringy nature of the temperature patterns depends on short angular distance behaviour of the anisotropy pattern, which is accurately represented in our results.

The formalism used gives an accurate estimate of the temperature shift induced by a segment of string for light rays which pass much closer than the horizon distance from the segment, but it overestimates the effect for light rays with larger impact parameters. Thus our maps should contain spuriously large temperature anisotropy for large angular wavelengths but should be fairly accurate for wavelengths smaller than the projected horizon size. Many of the qualitative features we are interested in here should not be affected by this long-wavelength component of the temperature pattern. We shall also see that even with the non-cosmological formalism there is a natural low-frequency cutoff at about the projected horizon size, which results from the coherence length of the strings themselves. Other

effects neglected by our technique are:

- (1) Temperature, velocity and potential perturbations at the surface of last scattering.
- (2) Sachs-Wolfe effect from decaying-mode potential perturbations between the surface of last scattering and us.
- (3) Anisotropies from baryonic perturbations at the last scattering surface.
- (4) Anisotropies from gravitational waves emitted by strings before last scattering.
- (5) Possible secondary anisotropies should the universe become reionized and from foreground sources.

We expect that (2), (3) and (4) should lead to smaller amplitude perturbations than those discussed here, although we have no space to justify these sentiments here. Effect (5) is very model-dependent and could be important or negligible. Effect (1) is probably the most important and its amplitude could be comparable to the one we are considering^{9,10}. If the universe does not become reionized we would expect that the coherence angle of this component of anisotropy would be fairly small ($\sim 10'$) because the surface of last scattering is fairly thin ($\Delta z_{ls}/z_{ls} \sim 0.1$). But if the universe is reionized then it is likely that $\Delta z_{ls}/z_{ls} \sim 1$ and the surface of last scattering would contribute only to longer wavelength ($\sim \Theta_{H_i}$) anisotropies. To see that a temperature pattern has sharp discontinuities, both the beam size and the beam throw must be smaller than the angular scale of the patchiness of the pattern. If the amplitude of effect (1) is large and is patchy on the scale of $10'$ (that is, no reionization) then it may be difficult to achieve sufficient detector sensitivity and sufficient sky coverage to satisfy these criteria and thus to obtain a statistically significant result. By ignoring effect (1) we are idealizing the calculation in such a way as to make the stringy character of the temperature pattern more evident.

The temperature pattern

The temperature pattern induced by the strings in the two boxes

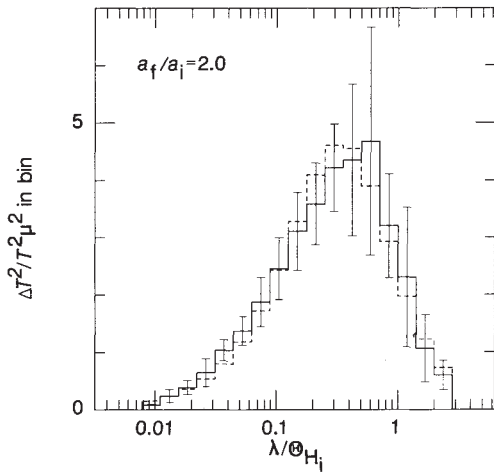


Fig. 3 The power $(\Delta T^2/T^2 \times (G\mu/c^2)^{-2})$, in various wavelength bins, in the anisotropy pattern produced as a string network expands by a factor of two. Θ_{H_i} refers to the initial angular size of the horizon. The error bars are the standard deviation about the mean for 12 different slices through a single simulation. The dashed line is an analytic fit.

placed end-to-end is shown in Fig. 1. As this image is the sum of the temperature patterns from one periodic box plus only one sixteenth of a second periodic box, the pattern presented is not itself periodic. The r.m.s. temperature fluctuation from the mean is $17 G\mu/c^2$ for this realization. Here μ is the string mass per unit length; G and c have their usual meanings. In Fig. 2, the projected position of the strings on the observer's past light-cone is superimposed onto the image of Fig. 1. The strings' positions and velocities on the past light-cone fully determine the temperature pattern⁸. Note that curves on which the temperature changes discontinuously correspond to the curves traced out by strings. But it is difficult to trace out a particular string from the temperature pattern because the magnitude and sign of the jump will change along the string as the string's velocity changes. Because of small transverse velocities, some strings which are quite apparent in Fig. 2 are indiscernible in Fig. 1. The temperature pattern may be described as a patchwork of hot (blue) and cold (red) regions, the sizes of which are determined by the coherence length of the velocity of the long strings. The string loops that are present in the simulation are generally very small (with some notable exceptions) and have little effect on the overall pattern. One can also see coherent patterns with wavelength much larger than the size of the patches. These can be shown to be produced by long strings at relatively low redshift, for which the projected horizon and coherence lengths are larger than for most other strings.

Angular power spectra

The r.m.s. response of any linear anisotropy measurement, when averaged over positions and orientations on the sky, is fully determined by the power spectra of the temperature pattern and the beam pattern. To determine which wavelength perturbations are most important we have divided each simulation volume into four slices. Each slice corresponds to the region swept out by the photons as the universe expands by a factor of two, during which time the horizon increases by only a factor of $\sqrt{2}$. We have Fourier-transformed the temperature pattern induced by the strings in each slice and scaled the wavelengths by the horizon size when the photons entered each slice. If our strings were truly demonstrating scaling behaviour throughout the simulation then these rescaled power spectra should show no systematic dependence, from slice to slice, on whether the slice came from early or late in our run. There is some apparent evolution in the string shapes with time, these being mostly related to the unrealistic initial conditions for the strings. When

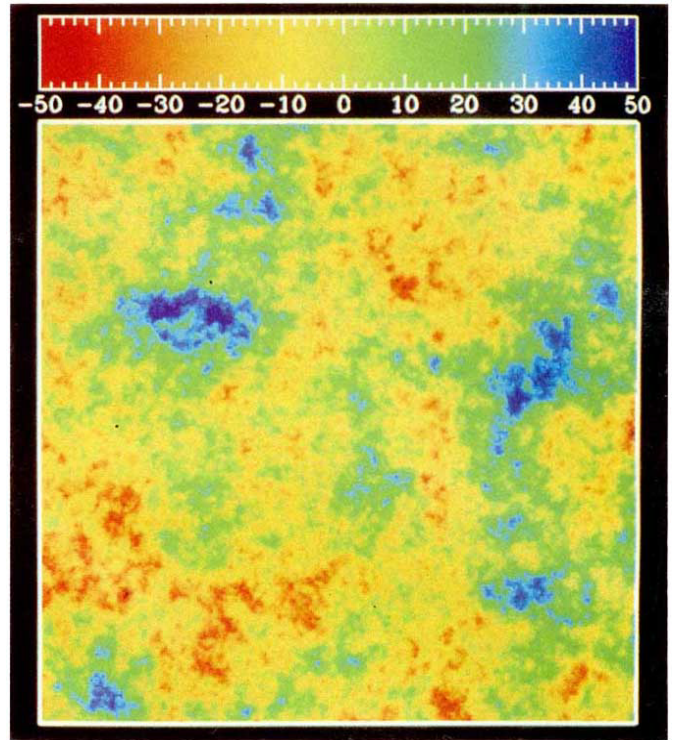


Fig. 4 $\Delta T/T$ with the same power spectrum as Fig. 1 but with random phases.

making the comparison of the power spectra, the most striking deviation from scaling is that when a given waveband corresponds to the size of the box the power carried by this waveband is enhanced. This is probably an artefact of the periodic boundary conditions. Because the longest angular wavelengths are suspect in any case, we omit the (0, 1) and (1, 0) and (1, 1) modes in subsequent discussion. Aside from these fundamental modes, no clear systematic evolution of the power spectra is manifest above run-to-run variations. Undoubtedly this is partially due to the small number of simulations, but we feel that for the purposes of anisotropy the strings are close enough to the scaling solution that this does not constitute a major inaccuracy in our method.

In Fig. 3 we present the average and standard deviation of the power in various wavelength bins for all of the twelve slices (four slices per direction, three directions). There is a clear peak at about half the horizon size which is the imprint of the coherence length of the strings on the temperature pattern they induce. There is also some evidence for convergence at both long and short wavelengths. Cosmological corrections of our formalism should cut off the long wavelengths more sharply, but given the cutoff that is already present it is not clear this will make much difference. The total power in wavelengths shorter than a wavelength λ_* is well fitted by the function

$$P_2(\lambda < \lambda_*, \Theta_{H_i}) = \frac{\Delta_2 T^2}{T} \left(\frac{\lambda_*^{1.7}}{(0.6\Theta_{H_i})^{1.7} + \lambda_*^{1.7}} \right)^{0.7} \quad (4)$$

where $\Delta_2 T/T \approx 6G\mu/c^2$. The quantity $\Delta_2 T/T$ is the r.m.s. temperature anisotropy from strings in the redshift interval $z-2z$. The binned power for this function is also plotted in Fig. 3.

The total mean square ΔT of the sum of adjacent slices is equal to the sum of the mean square ΔT of each individual slice to within about 5%, indicating that the temperature patterns in adjacent slices are relatively uncorrelated. Thus to determine the power spectra of anisotropy produced between z_{1s} and $2^{-n}z_{1s}$ it is a good approximation to simply add the power spectra given in equation (4) for each expansion factor of two, increasing

the wavelengths λ by $\sqrt{2}$ each time to account for the increasing horizon size:

$$P_2^N(\lambda < \lambda_*, \Theta_{H_i}) \approx \sum_{n=1}^N P_2(\lambda < \lambda_*, 2^{(n-1)/2} \Theta_{H_i}) \frac{\Delta_2 T^2}{T} \quad (5)$$

Thus we may estimate the total r.m.s. anisotropy from all wavelengths, obtaining

$$\frac{\Delta_{\text{tot}} T}{T} \approx \frac{\Delta_2 T}{T} \sqrt{\frac{\ln(z_{1s})}{\ln(2)}} = 19 \sqrt{1 - \frac{1}{3} \log_{10} \frac{1,000}{z_{1s}}} \frac{G\mu}{c^2} \quad (6)$$

Actual anisotropy experiments are only sensitive to a narrow range of angular wavelengths. We may use the approximate spectrum of equation (5) to estimate the expected r.m.s. signal for particular experiments. This is done by multiplying the power spectrum by a window function (which is the Fourier transform of the beam pattern) before integrating over Fourier space. We have done such an analysis for the Melchiorri *et al.* experiment¹¹, which reported a r.m.s. value of $\Delta T/T = 4 \times 10^{-5}$ at an angular scale of 6° . Because the signal could be due to galactic emission, we have used their 95% confidence limit of $\Delta T/T < 4.8 \times 10^{-5}$ to obtain $G\mu/c^2 < 5 \times 10^{-6}$. The main advantage of experiments at large angular scales (5° or more) is that most of the contribution to the fluctuations on these scales comes from small redshifts (< 30) and the limits they set are largely independent of the ionization history of the Universe.

If $z_{1s} \approx 1,000$, experiments on smaller angular scales may be able to set more stringent limits on the string tension. For instance, the analysis described above yields the limit $G\mu/c^2 < 2 \times 10^{-6}$ when applied to the measurement of $\Delta T/T < 1.5 \times 10^{-5}$ at 7 arcmin reported recently by the Caltech Owens Valley group¹². The problem with this analysis is that the experimentalists have assumed gaussian statistics in obtaining the upper limit on $\Delta T/T$ whereas the temperature pattern for strings differs appreciably from a gaussian pattern at small scales. We shall see below how the non-gaussian nature of the temperature pattern might substantially weaken the limits on $G\mu/c^2$ from small-angle experiments.

Non-gaussian temperature pattern

While the power spectrum of the temperature patterns is very useful, the temperature discontinuities and patchy appearance of the patterns require very specific phase correlations in the Fourier representation. These phase correlations make the interpretation of MBR anisotropy experiments more difficult, but they also provide a unique 'stringy' signature that will allow for actual detection of strings. To illustrate the effect of these phase correlations, we have Fourier-transformed the temperature maps that were combined to produce Fig. 1. We then changed the phase of each Fourier amplitude to a random number and combined the two maps in the same way as for Fig. 1. The resulting pattern is shown in Fig. 4. This second image has exactly the same power spectrum, but the phase coherence has been lost. The high-frequency modes do not interfere constructively with the low-frequency ones to produce sharp features, but give rise to many small-scale fluctuations. Another way to see the difference between random gaussian fluctuations and stringy ones is to examine one-dimensional scans of the two temperature maps (Figs 1 and 4). Figure 5 shows three such scans for each temperature map, which differ only in that the temperature patterns have been smoothed (in two dimensions) with different gaussian windows for each scan. The full width at half maximum (FWHM) for these scans are 0.04, 0.02 and 0.008 of Θ_{H_i} , or 4, 2 and $0.9 \times \sqrt{1,000/z_{1s}}$ arcmin. It is clear that a FWHM of 2 arcmin is quite sufficient to distinguish the flat plateaus and sharp jumps of the stringy temperature pattern from its gaussian counterpart. For $z_{1s} \approx 1,000$ this begins to be somewhat more difficult (although still possible) with a FWHM of 4 arcmin.

Figure 5 can also be used to illustrate how the power spectrum analysis discussed in the last section can give overly stringent

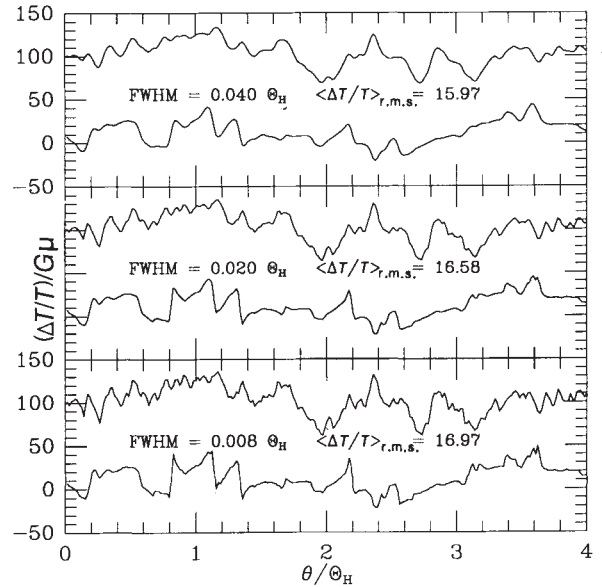


Fig. 5 Responses of three single-beam detectors for a linear scan across the string temperature map (Fig. 1). Displaced 100 units above is a scan through the random-phase temperature map (Fig. 4). The three detectors have FWHM beamwidths $4\sqrt{1,000/(1+z_{1s})}$ (top curves), $2\sqrt{1,000/(1+z_{1s})}$ (middle curves) and $0.9\sqrt{1,000/(1+z_{1s})}$ (bottom curves). The angular length of each scan is the same as the angular size of the two-dimensional maps, $7.2^\circ\sqrt{1,000/(1+z_{1s})}$.

limits on $G\mu/c^2$ when applied to small-scale experiments. The Caltech group¹² uses a 3-beam detector with a separation of 7 arcmin (or $0.06\Theta_{H_i}\sqrt{1,000/z_{1s}}$) between the beams. The FWHM for the Owens Valley detector is ~ 2 arcmin, so we can simulate their experiment by selecting regions of length $0.12\Theta_{H_i}$ from the middle graphs of Fig. 5 (assuming $z_{1s} \approx 1,000$). Because the short-wavelength power in the string pattern is concentrated in relatively rare jumps it is clear that the predicted distribution of detector responses is much broader than a gaussian. The Owens Valley group has used data from only seven independent spots on the sky to obtain their 95% confidence limit on the r.m.s. $\Delta T/T$. It is likely that this experiment would give a weaker limit on the r.m.s. $\Delta T/T$ of the non-gaussian patterns produced by strings.

Conclusions

We have produced a map of the expected MBR anisotropies produced by a network of cosmic strings and we have obtained the limit $G\mu/c^2 < 5 \times 10^{-6}$ on the single fundamental parameter describing cosmic strings. This limit is much firmer than that from gravitational radiation¹³⁻¹⁵ because the latter depend on the detailed properties of string loops, which are very uncertain⁵⁻⁷. It is also not far from the values of $G\mu/c^2 \approx 1-4 \times 10^{-6}$ that have been predicted from galaxy-formation consideration¹⁶⁻¹⁹. If the latter are accurate, then the MBR anisotropy from strings is likely to be observable in the next generation of anisotropy experiments. Should anisotropies be detected at this level, it should be possible to detect the stringy character of the anisotropy pattern if the anisotropies are really due to strings. One possible strategy for looking for the stringy features of an anisotropy pattern is to examine the anisotropy along a line of a few degrees with a detector having roughly the same parameters as the Caltech Owens Valley experiment¹² (a sensitivity of at least a few $\times 10^{-6}$ is probably required). Another possibility is to find candidate stringy jumps in the temperature pattern by an experiment with a resolution of a few tens of arcmin and then to examine such regions more closely with a higher resolution experiment.

In a future paper, we will examine strategies to search for the stringiness of an anisotropy pattern in more detail by running Monte Carlo experiments on our anisotropy map. We will also use Monte Carlo experiments to determine the proper limits on $G\mu/c^2$ from small-angular-scale experiments. Finally, we will also attempt a more quantitative estimate of other anisotropies

predicted by cosmic string models, which might interfere with the stringy nature of the anisotropies discussed here.

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Amplification and analysis of DNA sequences in single human sperm and diploid cells

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The use of the polymerase chain reaction for analysing DNA sequences in individual diploid cells and human sperm shows that two genetic loci can be co-amplified from a single sperm, which may allow the analysis of previously inaccessible genetic phenomena.

THE construction of genetic maps in higher organisms requires analysis of the progeny of selected matings or calculation of linkage relationships by pedigree analysis. In humans only the latter is possible. Using restriction fragment length polymorphisms (RFLPs), there has been significant progress towards the construction of a human linkage map (for review see ref. 1). To locate genes with known phenotypic effects relative to RFLP markers there has been a concerted effort to establish a panel of genetic markers at about 10 cM (1 cM = 1% recombination) intervals so that no gene will be further than 5 cM away from an RFLP (ref. 2). Pedigree analysis is thought to be able to measure genetic distances of about 1 cM encompassing about 1,000 kilobases (kb) of DNA with statistical reliability. The analysis of smaller distances requires such a large number of individuals from informative families that it is impractical. If the genotype of large numbers of individual sperm could be determined, the measurement of genetic recombination over shorter physical distances could be accomplished at a resolution far greater than that currently possible and without family studies. We have therefore attempted to define the conditions required to analyse DNA sequences in single cells using the polymerase chain reaction (PCR, refs 3 and 4). We studied single diploid cells and then single sperm from an individual heterozygous at two genetic loci found on non-homologous chromosomes. For each locus we determined which of the two alleles were present in any one sperm and analysed the independent assortment of the alleles at a single locus and the independent segregation of genes on non-homologous chromosomes.

Analysis in single diploid cells

We studied the human β -globin gene locus in individual diploid cells from two tissue culture cell lines. One was derived from a

homozygous individual for the sickle-cell mutation at codon six (β^S); the other was homozygous for the normal β^A allele. PCR primers that amplify a β -globin fragment containing codon six and allele-specific oligonucleotide probes (ASO) which can distinguish between these two alleles have already been described^{3,5}. We co-cultivated the cells homozygous for β^A and cells homozygous for β^S in the same tissue culture flask for several days. Individual cells from this mixture were drawn into a thin plastic pipette during observation under a phase-contrast microscope. Each individual cell was delivered into a PCR tube containing a lysis solution and, after incubation, PCR buffer containing deoxyribonucleotides, *Taq* DNA polymerase⁶ and a set of PCR primers that amplify the informative region of the globin gene was added. After DNA denaturation 50 cycles of amplification were performed⁶. Aliquots from each sample of amplified product were hybridized separately with the β^A and β^S probes after fixation to nylon membranes^{5,7}. The results of this co-cultivation experiment are shown in Fig. 1. Out of the 37 cells analysed, 84% hybridized with only one of the two allele-specific probes; 19 with the β^A probe and 12 with the β^S probe. None of the 12 control tubes, which received water instead of a cell, was positive, indicating that DNA contamination was insignificant. No sample hybridized with both probes, indicating that a single cell only was introduced into each tube and that DNA from lysed cells present in the co-cultivation mixture did not adhere to individual cells.

The amount of amplified β -globin gene product produced by PCR of a single cell was determined by comparing the intensity of the hybridization signal obtained with that from known amounts of plasmid DNA carrying the globin gene spotted on the same filter (Fig. 1). We estimate that, starting with a diploid amount of globin DNA (3.3×10^{-9} fmol), between 5 and 500 fmol of PCR product was produced in 50 cycles. This is equivalent to an average amplification ratio of 7.6×10^{10} with

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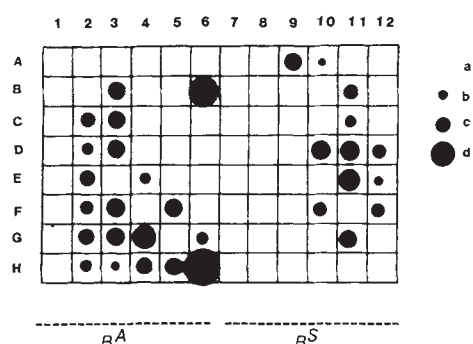


Fig. 1 PCR analysis of the β -globin gene in individual tissue culture cells. Each of two aliquots from the PCR products of a single cell were placed in the same row of the dot blot apparatus separated by six columns. Half of the filter was hybridized to the β^A ASO and the other to the β^S ASO. 1A–1H (7A–7H) and 2A, 2B (8A, 8B) were water blanks. 6H and 12H are aliquots of PCR product of purified β^A DNA. One, 3, 10 and 30 fmol of β^S gene containing plasmid were dotted as hybridization standards at positions a, b, c and d respectively. The remaining samples were individual tissue culture cells. After washing three times single cells were selected from a cell suspension ($1-3 \times 10^6 \text{ ml}^{-1}$) with a Zeiss phase-contrast microscope at $\times 100$ using a plastic needle with a 0.1-mm-diameter opening made by pulling a flamed disposable 1-ml-graduated plastic pipet. Each single cell sample was delivered into a 0.5 ml plastic microfuge tube containing 10 μl autoclaved distilled water. The cells were lysed using a slight modification of a published sperm lysis procedure¹⁶. The sample was adjusted to a final volume of 20 μl containing $1 \times \text{PCR}$ reaction buffer (ref. 6; 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM MgCl_2 , 0.1 mg ml^{-1} gelatin), 0.05 mg ml^{-1} proteinase K, 20 mM DTT, and 1.7 μM SDS. After one hour at 37 $^\circ\text{C}$, samples were heated to 85 $^\circ\text{C}$, and suspended in 100 μl PCR reaction mix containing $1 \times \text{PCR}$ buffer, 1 μM each oligonucleotide PCR primer, 187.5 μM each of dATP, dCTP, dGTP and dTTP, 100 ng *E. coli* DNA, 2 units of *Thermus aquaticus* thermostable DNA polymerase (Perkin Elmer-Cetus Instruments), and 60 μl mineral oil to prevent evaporation. The PCR reactions were carried out on a DNA Thermal Cycler (Perkin Elmer-Cetus Instruments). After heating at 95 $^\circ\text{C}$ for 10 min to ensure DNA denaturation, each cycle of PCR consisted of incubation at 95 $^\circ\text{C}$ for 15 s, 15 s incubation at 54 $^\circ\text{C}$ and a 1 min incubation at 72 $^\circ\text{C}$. After fifty cycles of PCR, dot blot analysis of 20 μl samples of the PCR reaction were carried out using β^S - and β^A -allele specific probes⁵.

an average efficiency per cycle of 65%. The precise extent of amplification may have been slightly lower if these cell lines contained more than two copies of chromosome 11. Considering the extent of amplification, elimination of all sources of possible contamination is critical to the success of these experiments.

Analysis in single human sperm

The genotype of single sperm derived from an individual heterozygous at the gene coding for the low density lipoprotein receptor (the *LDLr* gene), which has been localized to chromosome 19 (ref. 8), was analysed next. We adapted the detection of an *LDLr* polymorphism⁹ to PCR and ASO analysis using DNA sequence information (D. Russell, unpublished data), with the PCR primers and probes shown in the Fig. 2 legend. The size of the expected PCR product was 254 base pairs (bp).

Sperm were purified from a semen sample by centrifugation through a sucrose step gradient¹⁰ and stored for 8 months at -20°C . Individual sperm were drawn into a fine plastic needle under microscopic observation and delivered to a tube for lysis and amplification. In a series of experiments we analysed the *LDLr* genotypes in 80 individual sperm: typical results from one such experiment are shown in Fig. 2. Altogether 55% of

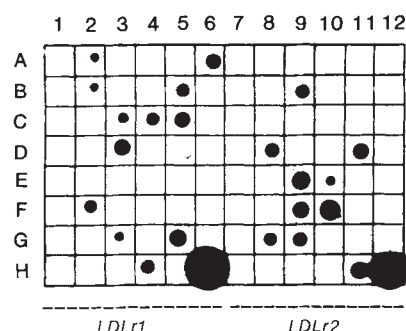


Fig. 2 PCR analysis of the LDL receptor locus of individual human sperm. 1A–1H (7A–7H) are water blanks. 6H and 12H are aliquots of amplified DNA from an *LDLr1/LDLr2* heterozygote. The remaining samples are from individual sperm. The organization of the samples on the dot blot is as in Fig. 1. Sperm were purified from semen by a modification of a published procedure¹⁰: 0.5 ml semen was mixed with 3 ml of 40% sucrose. The mixture was applied to the top of a sucrose step gradient made by adding 3.5 ml 90%, 70% and 50% (w/v) sucrose successively to a 15-ml graduated plastic tube (Falcon 2095). The sample was spun at 14,500g for two hours at room temperature. 0.5 ml of the interface between 70% and 90% sucrose was collected and applied to an identical sucrose gradient. This was repeated twice more. Single sperm were isolated in the same way as individual diploid cells using a sperm suspension at a concentration of $1 \times 10^5 \text{ sperm ml}^{-1}$. The sequence of the two primers used for PCR of the *LDLr* locus were 5'-AGTGCCAACCGCTCACAGG3' and 5'-CCTCTCACA-CCAGTTCACCT3'. The ASO for the *LDLr1* allele had the sequence 5'-AGGATATGGTCTCTTCCA3' whereas the *LDLr2* ASO had the sequence 5'-TGGAAGAGAACCATATCT3'. The PCR and dot blot analysis was performed as in Fig. 1 except that the final washes of the filters hybridized with the *LDLr* probes (and *HLA DQA* probes, see Fig. 3) were at 56 $^\circ\text{C}$.

the sperm gave a hybridization signal. Twenty-two carried one allele; 21 the other. Only one sample was positive with both probes. Sixteen additional control tubes which received all of the reagents but no sperm did not give any hybridization signal. The distribution of the two amplified alleles obeyed Mendel's law of independent segregation, indicating that the PCR reactions were initiated with a single meiotic product and that no contaminating diploid DNA sequences were present.

Independent assortment of chromosomes

We attempted to amplify simultaneously DNA sequences at two different loci on non-homologous chromosomes in a single sperm. Our sperm donor was heterozygous at the *HLA DQ- α* locus (*DQA*) on chromosome six, as well as at the *LDLr* gene. Primers for amplification of the first gene and probes to distinguish between allelic variants have been described previously^{5,11}. The predicted size of the PCR product was 242 bp. Initial experiments in which primers for both loci were present throughout the entire amplification experiment were unsuccessful, so we performed only the first 20 amplification cycles in the presence of both primer pairs. After this primary amplification, 1/50 of the reaction mixture was placed in a tube and diluted with a PCR solution containing the *HLA* primers only and another aliquot was diluted with a PCR mix containing the *LDLr* primers only. After an additional 45 cycles of amplification, part of each secondary reaction was hybridized to either of the two ASOs for that locus. A total of 150 individual sperm were analysed, in a series of such experiments (Table 1; representative data are shown in Fig. 3). Twenty-seven samples did not exhibit amplification of either locus, but we did detect hybridization signals in 123 samples (82%). In nine of these samples, two alleles from at least one of the two loci were

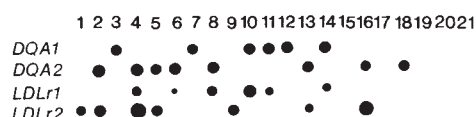


Fig. 3 PCR analysis of individual sperm simultaneously amplified with *HLA DQA* and *LDLr* primers and analysed with allele-specific probes for the *DQA* alleles 1 and 2 (*DQA1*, *DQA2*) and *LDLr* alleles 1 and 2 (*LDLr1*, *LDLr2*). Water blanks are in positions 19–21. Examples of sperm where one allele at each locus is amplified are shown in positions 2, 5, 6, 8, 10, 11, 13, 14 and 16. The remaining samples can be divided into those that amplified more than one allele at a locus (position 4), only one allele at one locus (positions 1, 3, 7, 9, 12, 17, 18) or neither allele (position 15). During the first 20 cycles both loci were amplified simultaneously with both sets of primers present at 1 μ M. A 2 μ l aliquot from each reaction was then added to each of two tubes with 100 μ l fresh PCR reaction buffer containing only one of the primer pairs. Forty-five additional PCR cycles were then performed.

detected and these samples were excluded from further analysis. These samples may have contained two sperm of different genotypes. On the other hand these nine samples might have resulted from non-disjunction events. The analysis of only two loci cannot distinguish between these possibilities.

Among the remaining 114 sperm, 96 could be typed at the *LDLr* locus with 45 having *LDLr1* and 51 having *LDLr2*. The two alleles segregate in the expected 1:1 ratio (Chi square = 0.375, $0.75 > P > 0.5$, 1 degree of freedom). Eighty-eight could be typed at the *DQA* locus: 53 had the *DQA1* allele, 35 the *DQA2*. The segregation of the *DQA* alleles with the expected 1:1 ratio is at the borderline of statistical significance (Chi square = 3.68, $0.1 > P > 0.05$, 1 degree of freedom). This could represent a statistical fluctuation, unequal amplification of the two *DQA* alleles resulting from base mismatches between the PCR primers and the *DQA* DNA sequence of our particular donor due to the extensive polymorphism of this locus, or some unusual genetic phenomenon such as segregation distortion.

Seventy of the 114 sperm (61%) that gave hybridization signals could be typed at both loci. The independent segregation of chromosome six and chromosome 19 should result in the equally frequent occurrence of the four possible gametes: *DQA1*, *LDLr1*; *DQA1*, *LDLr2*; *DQA2*, *LDLr1*; *DQA2*, *LDLr2*. We actually observed 21, 18, 14 and 17 of each type respectively; the difference is not statistically significant (Chi square = 1.43, $0.75 > P > 0.5$, 3 degrees of freedom). These results show that we can reliably and with reasonable efficiency simultaneously determine the genotype of individual sperm at two distinct genetic loci.

Among the 114 samples that gave a hybridization signal, 18 showed one of the two *DQA* alleles but an *LDLr* product could not be detected. Twenty-six sperm showed one of the two *LDLr* alleles but no amplification of the *HLA* locus. These sperm could be nullisomic for one of the two chromosomes we studied due to a non-disjunction event. There may also have been a failure to amplify one of the two loci. Because we only looked at a single locus on each chromosome we cannot distinguish between these possibilities.

The relative frequency of successful amplification of the *LDLr* and *DQA* loci in single sperm is approximately the same. Among 141 samples, 88 amplified *DQA* and 96 amplified *LDLr*. We are unable to calculate accurately the absolute frequency of success because we cannot be sure that every one of the 141 samples did in fact contain a sperm. As a result, we cannot use our data to calculate accurately the expected frequency of samples with a single allele amplified at both loci, at only one locus or at neither locus assuming that amplification at each locus is an independent event. Amplification of both loci may not be independent events if sperm lysis is not uniform from sample to sample. Thus the assessability of one of the target DNA

molecules to the PCR reagents might be positively correlated with the accessibility of the other. Improving the absolute rate of successful amplification of single sperm may depend upon improving lysis procedures, thereby enhancing target DNA accessibility.

Discussion

The analysis of the genotype of single sperm at the DNA level is a unique tool for the study of problems in human genetics considered intractable using current approaches. To date there have been no practical methods for accurate measurement of genetic distances of less than 1 cM. A significant advantage of the approach described here is that a large number of meiotic products can be examined from a single individual allowing determination of the recombination frequency between genetic markers which are physically very close. In conjunction with gel electrophoresis procedures for very large DNA fragments and chromosome-walking data it should be possible to measure the frequency of recombination between genetic markers whose physical distance apart is known precisely. This would allow the analysis of recombination frequency as a function of physical distance and a test of the conventionally accepted value of one per cent recombination per million base pairs¹² for specific chromosomal regions. Because it should be possible to obtain statistically significant data on recombination frequencies from a single individual, it should also be possible to determine for the first time whether different individuals have the same or different rates of recombination for the same interval.

The ability to measure recombination over short physical distances will be especially useful in the study of recombination hot spots. The effect that a hot spot can have on recombination between flanking markers depends upon how far away the flanking marker loci are from the ends of the hot spot. Thus a 1-kb DNA segment with a recombination potential which is 10-fold greater than normal DNA will have very little effect on recombination measured between markers that are 500 kb to either side of the hot spot. Pedigree analysis cannot measure recombination over the short intervals typical of many of the hot spot regions that have been deduced from population genetics data^{13–15}, given the number of informative families required and the effort involved in obtaining the data. With PCR, we can envisage typing as many as 500 meiotic products in a week. Decreasing the number of samples containing two sperm and increasing the efficiency of amplification of both loci simultaneously will be required for the highest resolution

Table 1 Amplification of sequences at two different loci

Total number of sperm examined	150
No signal	27
<i>DQA1</i> , <i>LDLr1</i>	21
<i>DQA1</i> , <i>LDLr2</i>	18
<i>DQA2</i> , <i>LDLr1</i>	14
<i>DQA2</i> , <i>LDLr2</i>	17
<i>DQA1</i>	14
<i>DQA2</i>	4
<i>LDLr1</i>	10
<i>LDLr2</i>	16
<i>DQA1</i> , <i>LDLr1</i> , <i>LDLr2</i>	2
<i>DQA2</i> , <i>LDLr1</i> , <i>LDLr2</i>	1
<i>DQA1</i> , <i>DQA2</i>	1
<i>LDLr1</i> , <i>LDLr2</i>	2
<i>DQA1</i> , <i>DQA2</i> , <i>LDLr1</i> , <i>LDLr2</i>	3
Controls	32
No signal	29
<i>LDLr1</i>	2
<i>LDLr2</i>	1

studies. In linkage experiments a fraction of the samples that contain two sperm and which have amplified only one of the two alleles at each locus will generate 'false recombinants'. We expect that the frequency of false recombinants can be minimized by careful attention to sperm isolation and the enhancement of sperm lysis and amplification efficiency. If very large numbers of sperm could be analysed with great reliability, some mutational events which cannot be analysed by conventional methods might eventually be studied.

Our ability to haplotype sperm at the DNA level will provide a fundamentally new approach to studying human recombination and may also be useful in determining the physical order of DNA polymorphisms which are so tightly linked that they cannot be resolved by additional family analysis. This may be especially significant in the case of random RFLPs tightly linked to disease-causing loci. The genetic distances between the random RFLPs could be accurately determined and the RFLPs ordered with respect to one another by three point crosses, provided that simultaneous amplification of 3 marker loci could be made efficient enough for single sperm experiments. Such fine structure maps might be of great value in attempts to locate the disease-causing locus itself. Of course because sperm do not exhibit disease phenotypes, an unknown disease locus cannot be directly mapped relative to polymorphisms in this way.

The analysis of single sperm in species that cannot be exten-

sively bred or have exceptionally long generation times may be the only practical way of making genetic maps for these species.

One immediate practical application of the analysis of individual sperm is in the area of forensic medicine. *HLA* typing for paternity determinations or identification of criminals is often hampered by the inability to determine the haplotype of the suspected individuals because this would require the analysis of close relatives. *HLA* analysis of individual sperm from a suspect would allow the linkage phase of the *HLA* markers to be unambiguously determined and thus increase the probability of inclusion or exclusion.

Finally, the ability to study DNA sequences in individual diploid cells will make it possible to study cell-to-cell variation in developmental processes involving DNA rearrangements or other genetic alterations. It is also likely that analysis of messenger RNAs in single cells would be possible if efficient reverse transcription could be carried out before PCR was initiated. Prenatal diagnosis on a single cell derived from a pre-implantation embryo resulting from *in vitro* fertilization is also conceivable.

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LETTERS TO NATURE

No cometesimals in the inner Solar System

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Ultraviolet measurements made by Voyager 2, apparently showing a rapid decrease in hydrogen Lyman- α emission with distance from the Sun, were taken by Donahue *et al.*¹ as evidence for a source of atomic hydrogen in the very local interstellar medium (VLISM). The suggested source¹ is a class of small comets, at solar distances of ~ 1 AU, that produce atomic hydrogen as their icy mantle is evaporated. This claim has been adduced as evidence for a theory² that the Earth is subject to a large influx of cometary material, significantly affecting atmospheric evolution. Here we analyse again the Voyager 2 data, and show that no source of hydrogen in the VLISM is required other than the inflow of neutral atoms; the original analysis was apparently flawed by an erroneous transcription of tabulated data (T. M. Donahue, personal communication).

Although Donahue *et al.*¹ claimed to find a cometary source of hydrogen, the estimated flux was $\sim$ seven orders of magnitude smaller than required by the theory of Frank *et al.*². But by postulating a different and more numerous kind of comet, Frank

et al. have claimed³ that the Voyager data, the most important direct evidence for small comets and the crucial data in obtaining limits on sources of volatile compounds such as H_2O , can be made to agree with their theory.

The observations in question are obtained with Voyager 2 shortly after launch¹. Figure 1 shows the observing geometry looking down on the north pole of the Solar System. A number of observations, in a direction approximately downstream with respect to the inflowing VLISM neutral gas, were obtained with the spacecraft located near 1 AU. Further measurements were obtained with the spacecraft located at $r_0 \sim 1.3$ AU and beyond, with all observations approximately downstream as shown in Fig. 1. The analysis of these data by Donahue *et al.*¹ indicated that the measurements near 1 AU in a direction almost tangential to the Earth's orbit demonstrated intensities in excess of a normal VLISM model. The excess near 1 AU was attributed to the influx of cometesimals. We argue that the stronger signal obtained near 1 AU is simply a consequence of observing geometry.

The search for a local source of hydrogen in the Voyager data was limited by Donahue *et al.*¹ to observations in the VLISM downstream direction (Fig. 1). The observations obtained with the spacecraft positioned near 1 AU were necessarily at a high angle ($\beta \approx 90^\circ$; see Fig. 1) to the antisolar direction because the spacecraft was positioned in the vicinity of 0° right ascension (α) (Fig. 1). Later observations with the spacecraft positioned near 2 AU were obtained with a viewing direction more closely aligned with the antisolar direction. A non-negligible fraction

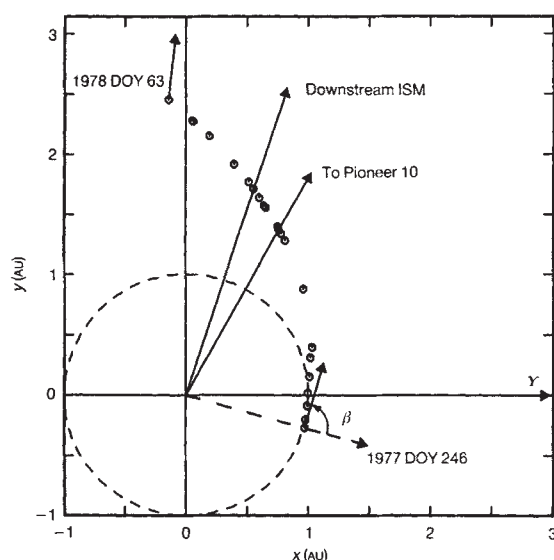


Fig. 1 Voyager 2 spacecraft locations and pointing directions as viewed from the north pole of the Solar System. Y indicates the $\alpha = 0$ reference direction. The positions refer to the data used by Donahue *et al.*¹. The observational direction for the Voyager ultraviolet spectrograph is shown for 3 September 1977 (DOY 246) and 4 March 1978 (DOY 63). The direction of the Pioneer 10 spacecraft position (at ~ 15 AU) and mean observational direction (dashed arrow) during this period, and the direction of the flow of the neutral VLISM gas are also indicated. DOY = day of year. The dashed arrow denotes the antisolar direction for the first Voyager observation used in the analysis.

of the observed hydrogen Lyman- α ($Ly\alpha$) signal is due to scattering from the inner cavity region. If one approximates the hydrogen distribution in this region as a constant density, this fraction is roughly proportional to $(\beta/\sin \beta)$. Because the observational angle β approaches 90° in the data obtained near 1 AU, the signal produced in the downstream direction is significantly larger than expected relative to the measurements made later near 2 AU, where $\beta \sim 0^\circ$. In other words, the data

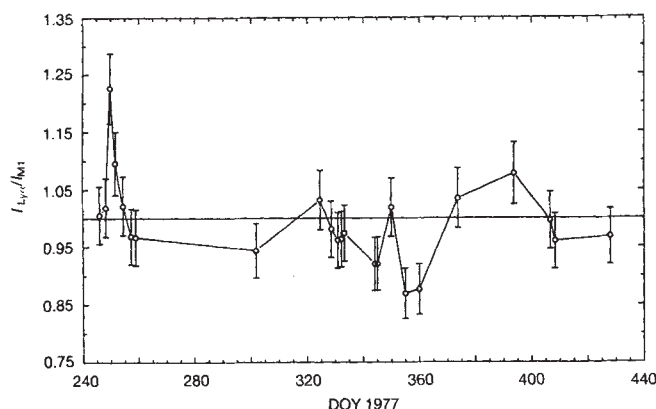


Fig. 2 The ratio of the $Ly\alpha$ intensity measured by Voyager 2 ($I_{Ly\alpha}$) relative to prediction of the VLISM model of Ajello *et al.*⁴ (I_{M1}), using the data obtained at points shown in Fig. 1. The ratio $I_{Ly\alpha}/I_{M1}$ is normalized to 1.0 averaged over the data points for 28 January to 4 March 1978 (DOY 393–428). The model includes the variation of the solar flux as defined by the Pioneer 10 data shown in Fig. 4. The mean ratio over the period 3–15 September 1977 (DOY 246–258) is $I_{Ly\alpha}/I_{M1} = 1.04 \pm 0.09$, compared to a mean value $I_{Ly\alpha}/I_{M1} = 1.00 \pm 0.05$ for the period 28 January to 4 March 1978 (DOY 393–428). Note that here DOY refers to days from 1 January 1977.

show a deceptively large signal near 1 AU because of observing geometry. Figure 2 shows the calculated ratio of the Voyager 2 intensity data, $I_{Ly\alpha}$, to the predicted signal based on the model of Ajello *et al.*⁴, I_{M1} , after correction for the variation in the solar $Ly\alpha$ flux. The ratio is normalized to $I_{Ly\alpha}/I_{M1} = 1.00 (\pm 0.05)$ in the averaged data points for 28 January to 4 March 1978 (days of year (DOY) 28–63), corresponding to a heliocentric distance $r_0 \geq 2.0$ AU. The data obtained in the time span 3–15 September 1977 (DOY 246–258) corresponds to a spacecraft position near $r_0 = 1.0$ AU. The value of $I_{Ly\alpha}/I_{M1}$ during this latter period scatters around the value 1.04 ± 0.09 ; this scatter appears to be limited by our ability to accurately define the solar flux during the integration time of the measurement. But the scatter in the $I_{Ly\alpha}/I_{M1}$ values in Fig. 2 is generally consistent with our estimated 1σ statistical error of $\pm 4\%$. The Ajello *et al.*⁴ model is based on the Thomas⁵ spatial structure of atomic hydrogen determined by assuming the only source of neutral gas to be the inflowing VLISM. Figure 3 shows a plot of Voyager intensity data against model predictions as a function of spacecraft radial position. The model calculation includes a correction for variation in solar $Ly\alpha$ flux defined by the Pioneer 10 data shown in Fig. 4. We conclude that the Ajello *et al.*⁴ model accounts for the data within errors.

As stated by Donahue *et al.*¹, the major uncertainty in establishing the observed distribution is the determination of the appropriate value of solar flux ($\mathcal{F}_0 Ly\alpha$) used for normalization. We have used the value obtained from Pioneer 10 (P10) $I_{Ly\alpha}$ data. The P10 spacecraft was located at $r_0 \approx 15$ AU and $\alpha \approx 61^\circ$ during this period and we have applied no phase correction to the data. Figure 4 shows a plot of the P10 solar reference data corrected for a r_0^{-1} variation in signal ($I_{Ly\alpha} r_0$), in comparison with predicted signal $\mathcal{F}_0 Ly\alpha$ at Earth based on He-10,830-Å equivalent-width measurements (see Donnelly *et al.*⁶ and Skinner *et al.*⁷). Using either data set to obtain $\mathcal{F}_0 Ly\alpha$ produces equivalent results in Fig. 4 after appropriate phase correction. The short-term variability in $\mathcal{F}_0 Ly\alpha$ indicated in the Atmospheric Explorer-E (AE-E) data used by Donahue *et al.*¹ does not agree particularly well with either data set in Fig. 4, and we regard the AE-E data as unreliable⁸. Nevertheless, reduction of the results using the AE-E data produces the same conclusion as we have obtained above, indicating that using any of these data sets to define $\mathcal{F}_0 Ly\alpha$ does not alter the basic conclusion, namely that there is no evidence of a measurable local source of atomic hydrogen in the Voyager data.

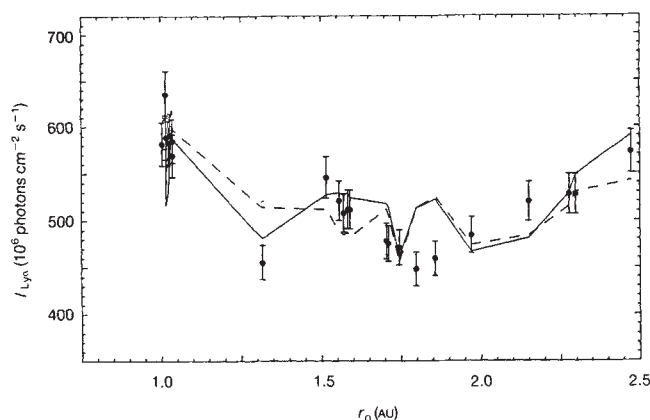


Fig. 3 Voyager 2 intensities for the data examined by Donahue *et al.*¹ as a function of spacecraft heliocentric radial position (solid circles), compared to normalized VLISM model calculations using the Ajello *et al.*⁴ model (solid line) and a simplified analytic approximation (dashed line). The model calculations are corrected for the solar $Ly\alpha$ flux variation as defined by the Pioneer 10 data (Fig. 4).

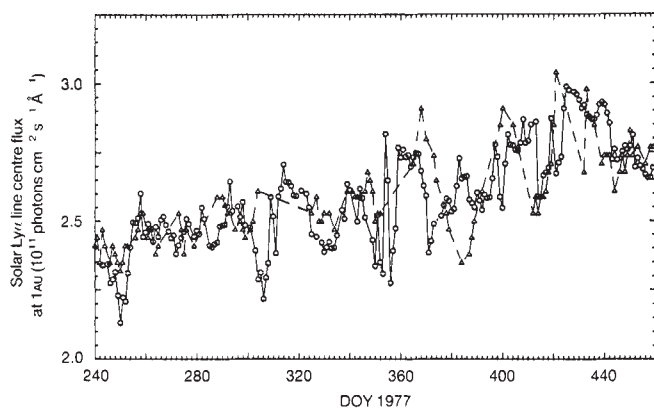


Fig. 4 Variation of the line centre solar Ly α flux as defined by the Pioneer 10 measurements of reflected solar radiation (open circles connected by a solid line) and measured equivalent width of the He-10,830-Å solar absorption feature (open triangles connected by a dashed line) over the period 28 August 1977 to 5 April 1978 (DOY 1977 240–460). The P10 data is proportional to the product $I_{\text{Ly}\alpha} r_0$. The P10 spacecraft location is in the range $r_0 = 13.5$ – 15.3 AU during this period. The data are normalized to SME reference data during 1982, with a conversion to predicted line centre flux by the factor $I_\lambda = 0.961 I$ (photons $\text{cm}^{-2} \text{s}^{-1} \text{\AA}^{-1}$). Solar line shape is assumed constant.

The results obtained here derive mainly from the observing geometry; the sensitivity to VLISM model parameters is minimal. In fact, the Voyager 2 results can be approximated using

$$I_{\text{Ly}\alpha} = g_e r_c^2 n_c \left(\frac{\beta}{r_0 \sin \beta} \right) + I_c \quad (1)$$

where g_e is the Ly α g value at distance $r_c = 1$ AU from the Sun, n_c is a characteristic inner cavity H I density, r_0 is the spacecraft radial distance, β is the angle between the line of sight and the antisolar direction and I_c is a constant intensity due to backscattering at or beyond some characteristic ionization cavity boundary. The model represented by equation 1 corresponds to the Donahue *et al.*¹ model with their parameter $\gamma = 2$. The model (dashed line) shown in Fig. 3 corresponds to $n_c = 0.008 \text{ cm}^{-3}$ and $I_c = 3.49 \times 10^8 \text{ photons cm}^{-2} \text{s}^{-1}$. The atomic hydrogen distribution in the calculation simply uses a step function H I distribution as an approximation to the detailed Thomas⁵ calculation.

We conclude that there is no systematic evidence for a local source of atomic hydrogen in the Voyager data. If such a source were present at 1 AU, it could have a Ly α emission rate no larger than $I_{\text{Ly}\alpha} \approx 20$ rayleighs, a factor of eight below the value obtained by Donahue *et al.*¹. Donahue (private communication) has obtained a similar result using the Thomas⁵ tabulated VLISM model and AE-E solar reference data.

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The oxygen isotope effect in $\text{Ba}_{0.625}\text{K}_{0.375}\text{BiO}_3$

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The nature of the superconducting pairing mechanism in the high-temperature superconducting oxides is still an open question. Shortly after the discovery of these materials, several groups began an intensive study of the oxygen isotope effect. For a monatomic conventional (BCS) superconductor, the transition temperature T_c is proportional to $M^{-\alpha}$, where M is the mass of the atom and $\alpha = 0.5$, assuming no anharmonicity for the phonon modes and no Coulomb interactions. For a multicomponent material, $T_c \propto M_i^{-\alpha_i}$ with an α_i defined for each ion with mass M_i . Thus, each element contributes to the overall α (which would still equal 0.5) with a weight dependent on the structure of the phonon modes that are important for superconductivity. Because the $^{16}\text{O}/^{18}\text{O}$ mass ratio is much smaller than the available mass ratios for the isotopes of the other atoms in these materials, the oxygen contribution to α (α_{ox}) should be the largest and thus in principle the easiest to measure. A zero or very small oxygen isotope effect would thus imply an unconventional pairing mechanism, although the suppression of α in normal phonon-mediated superconductors is common. A large value of α (>0.3) indicates conventional (BCS) superconductivity. $\text{Ba}_{1-x}\text{K}_x\text{BiO}_3$ was recently found to be superconducting¹, with $T_c \approx 30$ K. Unlike the planar cuprate superconductors, this material is cubic. Here we report on the ^{18}O isotope effect for $\text{Ba}_{0.625}\text{K}_{0.375}\text{BiO}_3$. We find that this material has a large isotope effect, indicating that the pairing mechanism is conventional (phonon-mediated).

Transition temperatures for $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$ (with a nominal T_c of ~ 38 K) have been found to decrease by 0.3–1 K on substitution of ^{18}O (refs 2–4), which implies a variation in α_{ox} of 0.1–0.37, with a most probable value of 0.2. T_c suppressions of 0–1 K have been reported for $\text{YBa}_2\text{Cu}_3\text{O}_7$ (refs 4–9), giving a much lower value of α_{ox} (0–0.05) than in $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$ because of the much higher T_c (91 K) of the former. These large variations in reported α should not be unexpected because for these materials the shift in T_c , ΔT_c , is much less than the superconducting transition width, making the determination (and even the criterion for the determination) of ΔT_c difficult. Synthesizing these materials with sample-to-sample variations that are less than the observed isotope shift is also difficult. But despite large variations in the reported oxygen isotope effects there is no doubt that α_{ox} for $\text{YBa}_2\text{Cu}_3\text{O}_7$ is much smaller than that for $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$.

The isotope effect has been measured recently for $\text{BaPb}_{1-x}\text{Bi}_x\text{O}_3$ ($T_c \approx 13$ K for $x = 0.25$) and was found to be large⁴, indicating that pairing is phonon-mediated in this material. From the results in ref. 4 ($T_c = 11$ K, $\Delta T_c = 0.5 \pm 0.1$ K, 60% ^{18}O substitution) α_{ox} is calculated to be 0.6 ± 0.1 . This value is unrealistically large, but if 100% substitution is assumed instead, more reasonable values (0.4 ± 0.1) are obtained. It is interesting that the magnitude of the T_c suppression appears to remain constant for these three oxide systems, leading to a monotonic decrease in α_{ox} as T_c increases. This has led to speculation that in these materials there is a continuous transition from phonon-mediated superconductivity (for low-temperature superconductors) to a dual phonon–electronic boson mechanism (for example, phonon–plasmon) for materials with high transition temperatures¹⁰. Thus, suppression of the isotope effect could be a measure of the non-phonon contribution to superconductivity. If α_{ox} scales with T_c , we would expect an

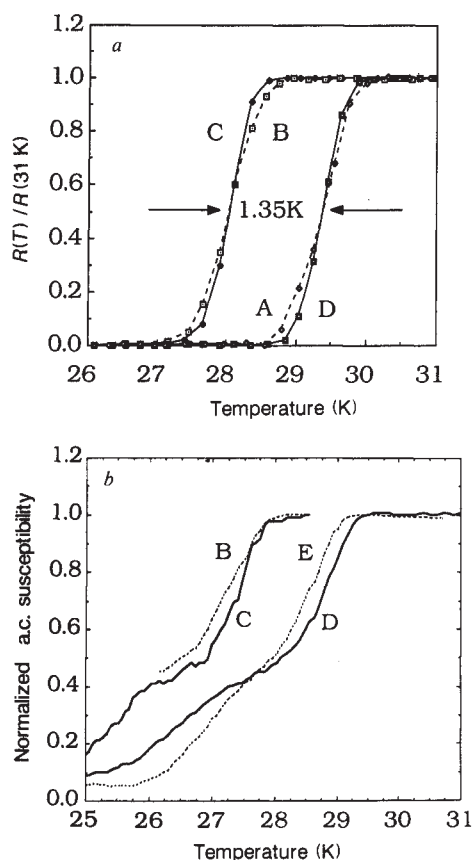


Fig. 1 Normalized measurements of the superconducting transition for $\text{Ba}_{0.625}\text{K}_{0.375}\text{BiO}_3$ samples after successive exchanges in either $^{16}\text{O}_2$ or $^{18}\text{O}_2$. Curve A is for the starting material after one $^{16}\text{O}_2$ exchange. Curves B and C are for one and two subsequent $^{18}\text{O}_2$ exchanges, respectively. Curve D is after two further $^{16}\text{O}_2$ exchanges. *a*, d.c. resistivity. *b*, a.c. susceptibility.

isotope effect for $\text{Ba}_{1-x}\text{K}_x\text{BiO}_3$ that is much reduced relative to $\text{BaPb}_{1-x}\text{Bi}_x\text{O}_3$ and is close to that found for $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$.

All isotope exchange experiments were done with material from one pellet synthesized using the method reported previously¹¹⁻¹². The starting composition of this material was $\text{Ba}_{0.625}\text{K}_{0.375}\text{BiO}_3$. Several pieces of this material (~100 mg in mass) were broken from the master pellet and placed in a gold boat contained in a quartz furnace tube. These pieces were reduced in a flowing Ar stream by heating the furnace to 700 °C at 2 °C min⁻¹. The furnace was held at this temperature for 1 h and then cooled to room temperature at 2 °C min⁻¹. The system was then evacuated and filled with either $^{16}\text{O}_2$ (Alfagaz UHP) or $^{18}\text{O}_2$ (Isotec, Inc. 99.1 atomic %) to 700 torr. The furnace was then heated to 450 °C at 2 °C min⁻¹, held for 0.5 h and cooled to room temperature at 1 °C min⁻¹. This firing sequence constitutes one exchange cycle. After each cycle (700-°C Ar firing followed by 450-°C $^{16}\text{O}_2$ - or $^{18}\text{O}_2$ -anneal) one small piece was removed for analysis. All remaining pieces were then subjected to the next cycle. As the system is closed during oxygen exchange, the system pressure increased to 825 torr at 450 °C. T_c is expected to vary with oxygen vacancy concentration, which will depend, in turn, on the temperature and oxygen pressure during firing. With the thermal cycle and oxygen pressure described above, our previous work indicates that the vacancy content should be very small. The above firing sequence was always followed exactly to ensure that any small, residual vacancy content remained constant.

The transition temperatures were measured using both a.c. susceptibility and d.c. resistivity. For the a.c. measurement, temperature was measured with a calibrated carbon-glass thermometer. For resistivity measurements (four-lead d.c. using

Ag-painted contacts) temperature was determined with a calibrated Si diode. The onset of T_c was ~0.3 K higher for the resistivity measurement than from susceptibility for the same sample. The exchange fraction was determined using thermogravimetric analysis (TGA) with a Perkin Elmer TGA 7.

During initial work with this system a major difficulty was encountered during the $^{18}\text{O}_2$ exchange cycles. The material was a deep blue colour after the $^{16}\text{O}_2$ exchange but became somewhat brown during the $^{18}\text{O}_2$ treatment. Susceptibility measurements still showed bulk superconductivity but resistivity measurements were difficult because the samples had large normal-state resistivities. The resistivity became zero only at low measuring currents, indicating a poorly connected superconducting sample. The problem was traced to the presence of H_2 in the $^{18}\text{O}_2$ (~0.8 atomic %). This chemically reduced the surface of the grains in the material, leading to a thin, high-resistance surface layer. H_2 was removed from the $^{18}\text{O}_2$ with a Pd catalyst, with subsequent distillation to separate the generated water. After this step, the room-temperature resistivities of the ^{16}O - and ^{18}O -containing samples were the same (~50 mΩ cm).

Figure 1a shows the d.c. resistivity for four samples with different firing sequences. Curve A shows the transition for a virgin sample subjected to one $^{16}\text{O}_2$ firing. T_c is reduced dramatically after just one exchange by $^{18}\text{O}_2$ (curve B) and a second exchange slightly sharpens the transition (curve C). The remaining pieces were then treated again with $^{16}\text{O}_2$. This is a crucial test of the observed isotope effect, because other structural changes might have occurred during the multiple firings (such as K loss from the superconducting phase). Curve D shows a sample after two $^{16}\text{O}_2$ cycles; this has become nearly identical to the starting material, indicating that the 16-18 exchange is totally reversible and that the sample was left structurally intact during the exchange reactions.

The a.c. susceptibility for these samples is shown in Fig. 1b. The curves were normalized so that the total signal change between 10 and 30 K was set equal to one. The suppression of T_c was consistent with the resistivity data, although for all the samples a low-temperature tail is present. This was not observed in the resistive transitions as this method measures only about 15% of the sample (zero resistance occurs at the percolation threshold for the highest- T_c material) whereas the susceptibility monitors the entire sample. The low-temperature tail, which was much larger in samples treated in the H_2 -contaminated $^{18}\text{O}_2$, is probably due to some residual moisture present in the system leading to a surface layer with structural disorder, such as oxygen vacancies or K nonuniformity.

The resistively measured ΔT_c is taken at 50% of the resistive transition between curves C and D of Fig. 1a. The T_c suppression is 1.35 ± 0.05 K and remains relatively constant throughout the entire resistive transition. The T_c suppression from the susceptibility data, measured from the onset to the 60% point (where the foot starts), is 1.4 ± 0.1 K for the same two samples (C and D). In the region below 60%, ΔT_c varies from 1 to 1.3 K. If we ignore the a.c. data below 60% a conservative value for the T_c suppression is 1.4 ± 0.1 K.

The total ^{18}O exchange fraction is the sum of two parts: the first is due to the filling of the oxygen vacancies introduced during Ar firing and the second is the normal 16-18 exchange that occurs during $^{18}\text{O}_2$ firing. During Ar firing a trivalent material is formed with the stoichiometry $\text{Ba}_{0.625}\text{K}_{0.375}\text{BiO}_{2.31}$ (ref. 12). Thus, the direct filling of vacancies will give an exchange fraction of 23%. Further exchange depends on the ratio of amount of oxygen in the system to that in the sample. Assuming equilibrium is reached, and using a conservative value for the exchange volume, we estimate that a single cycle will give an 85% exchange fraction.

Figure 2 shows the TGA for an ^{18}O sample after two $^{18}\text{O}_2$ exchange cycles. This sample was placed in the TGA apparatus with either flowing N_2 or O_2 and cycled using the same temperature program as used for the exchange experiments. The

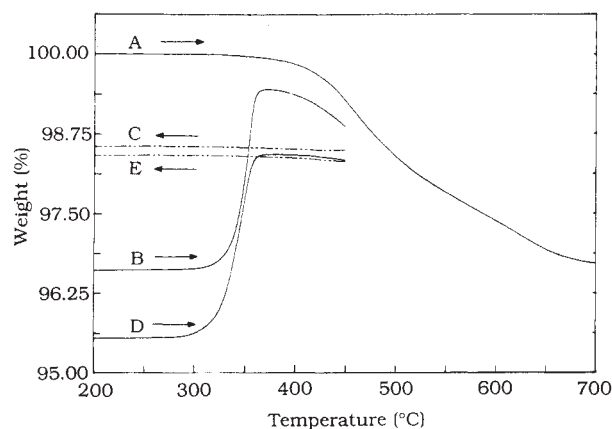


Fig. 2 TGA curves for a $\text{Ba}_{0.625}\text{K}_{0.375}\text{BiO}_3$ sample after two $^{18}\text{O}_2$ exchanges. Curve A shows the initial heating in N_2 (the nitrogen cool to RT is not shown). Curves B and C show the following heating and cooling in flowing oxygen. After a subsequent cycle to 700°C in N_2 (not shown for clarity) the sample is again cycled in O_2 (curves D and E). The arrows indicate the temperature scan direction.

solid curve A is for the initial 700°C N_2 firing (the N_2 cooling curve is not shown since the weight remains constant during cooling). The system is then flushed with O_2 and heated to 450°C at 2°C min^{-1} (curve B). The sample is held at 450°C for 30 min and cooled to room temperature (curve C). The entire cycle (N_2 at 700°C and O_2 at 450°C) is repeated and curves D and E show the oxygen heating and cooling part of the cycle. The decrease in weight for curve B above 375°C (after the vacancies are filled) is due to the normal exchange of ^{18}O by ^{16}O during heating. During the second O_2 firing this curvature is reduced, indicating that ^{18}O has been removed from the sample. From the total weight loss the stoichiometry of the sample is determined to be $\text{Ba}_{0.625}\text{K}_{0.375}\text{Bi}^{18}\text{O}_{2.89(5)}\text{O}_{0.11(5)}^{16}$, giving an exchange of $96 \pm 3\%$. For simplicity we assumed 100% exchange to calculate α_{ox} for our samples. Using $T_c = 29.4\text{ K}$ (50% resistive transition) and $\Delta T_c = 1.4 \pm 0.1\text{ K}$, we find $\alpha_{\text{ox}} = 0.41 \pm 0.03$.

This large value of α_{ox} shows that among the oxide superconductors α_{ox} does not scale with T_c . Both $\text{Ba}_{0.625}\text{K}_{0.375}\text{BiO}_3$ and $\text{BaPb}_{1-x}\text{Bi}_x\text{O}_3$ have large values of α_{ox} (0.4–0.5) even though the T_c are over a factor of two different. Both of these materials appear to be phonon-mediated BCS superconductors. $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$ has a T_c only $\sim 8\text{ K}$ above that of $\text{Ba}_{0.625}\text{K}_{0.375}\text{BiO}_3$ and yet α_{ox} for the former material is only half that of the latter. One could argue that in this temperature range a dual mechanism sets in which rapidly decreases α_{ox} as the phonon-mediated contribution rapidly decreases.

It is important to note that the bonding in the Bi–O systems is quite different from the Cu–O materials. The Cu–O bonding is strongly d in character while the Bi d -orbitals are filled and are far below the Fermi surface. For both of the Bi–O superconductors, bands formed from the Bi(6s)–O(2p) orbitals are located at the Fermi surface¹³, whereas the Cu(3d)–O(2p) bands are at the Fermi surface in the Cu–O superconductors. Strong d -bonding with narrow d -bands intersecting the Fermi surface is known to lead to reduced isotope effects in transition elements and intermetallic compounds. Garland¹⁴ has shown that the reduced isotope effect in these materials is due to the Coulomb interaction of d -electrons at the Fermi surface. The reduced isotope effect in $\text{La}_{1.85}\text{Sr}_{0.15}\text{CuO}_4$ relative to $\text{Ba}_{0.625}\text{K}_{0.375}\text{BiO}_3$ may not, therefore, be indicative of the onset of an exotic mechanism but may be instead simply the effect of differences in electronic structure at the Fermi surface.

It would be interesting if other, higher- T_c non- d -bonded superconductors could be found. The isotope effect in these materials could possibly delineate the temperature range of phonon-mediated superconductivity.

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Growth and structural characterization of superconducting $\text{Ba}_{1-x}\text{K}_x\text{BiO}_3$ single crystals

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Superconductivity near 30 K was discovered recently in a copper-free phase, $\text{Ba}_{1-x}\text{K}_x\text{BiO}_3$ (refs 1, 2). This material has structural and electronic properties in common with both the cuprate high-transition-temperature (high- T_c) superconductors³ and with $\text{BaPb}_{1-x}\text{Bi}_x\text{O}_3$, which has a T_c of $\sim 12\text{ K}$ (ref. 4). Here we report the growth and structural characterization of single crystals of $\text{Ba}_{1-x}\text{K}_x\text{BiO}_3$. A maximum T_c of 30.5 K is found from d.c. susceptibility measurements for crystals of approximate composition $\text{Ba}_{0.6}\text{K}_{0.4}\text{BiO}_3$. The structure for a crystal with $x = 0.37$ is determined by X-ray diffraction and is found to be a simple cubic perovskite with $a = 4.2869$ (6) Å. A commensurate lattice distortion occurs for $x = 0.04$, which is identified as a distortion of the BiO_6 octahedra. Effects of structural distortions and concomitant changes in electronic properties in this non-magnetic system may contain clues to the role of phonons and electronic interactions in the microscopic pairing mechanism of high- T_c superconductors.

Both the structure and transport properties of $\text{Ba}_{1-x}\text{K}_x\text{BiO}_3$ are a sensitive function of x . For $x = 0$, when the formal oxidation state of Bi is +4, the material is insulating and the structure is a monoclinic distortion of the ideal perovskite structure^{5–7} such that there are two distinct charge-ordered Bi sites. For $x = 0.4$, the cubic structure is undistorted^{8,9} and all Bi sites are equivalent both at room temperature and at 14 K (R. M. Fleming, P. Marsh, R. J. Cava and J. J. Krajewski, preprint). An orthorhombic distortion of the cubic perovskite has been reported⁸ for non-superconducting compositions with $x \leq 0.25$. Crystals of composition $\text{Ba}_{0.87}\text{K}_{0.13}\text{BiO}_3$, grown from a KOH flux, were reported to be cubic but not superconducting⁹. Here we describe the use of dry KOH melts for the growth and structural characterization of $\text{Ba}_{1-x}\text{K}_x\text{BiO}_3$ single crystals of various x .

Potassium hydroxide is a classical flux for the high-temperature solution growth of refractory oxides^{10–12}. Wignacourt *et al.*⁹ have grown cube-shaped black crystals of $\text{Ba}_{1-x}\text{K}_x\text{BiO}_3$ with $x = 0.13$ by dehydration of $\text{KOH} \cdot n\text{H}_2\text{O}$ melts

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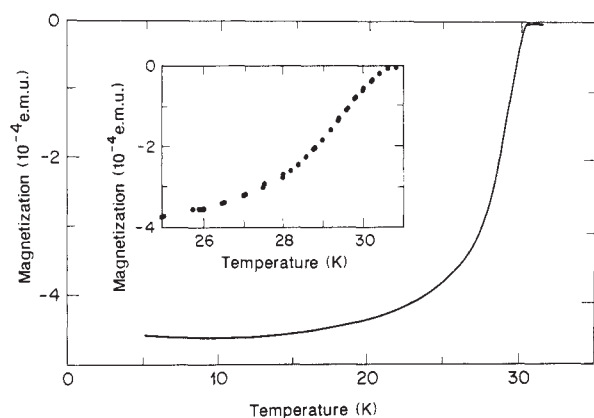


Fig. 1 Temperature dependence of the d.c. magnetization for a collection of crystals of composition $\text{Ba}_{0.6}\text{K}_{0.4}\text{BiO}_3$. Inset shows the transition region in more detail.

containing $\text{Ba}(\text{OH})_2$ and Bi_2O_3 . Crystals were small, a few tens of microns on an edge, but were suitable for a single-crystal structural determination, and were not superconducting (as expected on account of the low K content). By using dry KOH (dried by heating at 150°C *in vacuo* for 1–3 days), we have been able to vary the potassium substitutional doping level and have produced crystals of up to 0.3 mm on an edge. Dry KOH increases the basicity of the melt¹³ and thus promotes oxidation (K substitution). Gold or platinum crucibles were typically used, although vitreous carbon and silver were also employed successfully. Growth conditions and pseudo-cubic lattice constants obtained from X-ray powder data are listed in Table 1. Any distortions from cubic symmetry were unresolved in the powder data.

Crystal habits vary from flat platelets for low levels of potassium to regular black cubes which superconduct at 30.5 K. Visual observation of one crystal-growth run revealed that solute dissolution occurs near 425°C and crystal growth occurs over a narrow temperature range near 400°C just before melt solidification. Crystal size and yield were strongly dependent on solute concentration. No crystals were obtained at low solute concentrations and the largest crystals were obtained at solute concentrations of ~ 40 wt% KOH (~ 20 mol% solute). Crystals are of sufficient size for X-ray structural determinations and resistivity or magnetization measurements but are too small for many other studies.

Because of the small size of the individual crystals, transition temperatures for a collection of crystals from a single batch

Table 1 Crystal growth parameters

Batch	Ba/Bi	Wt% KOH	T_{max}	Atmosphere	T_c	a (Å)
1	0.48	80	450	O_2	30	
2	0.77	80	410	O_2	28	4.286 (4)
3	0.78	75	430	O_2	0	4.300 (3)
4	1.00	80	430	O_2	22	4.308 (4)
5	0.83	80	400	air	29	4.278 (6)
6	0.52	75	450	air	30	
7	0.25	40	430	air	30.5	4.277 (7)
8	0.6	80	475	air	30.5	4.287 (6)
9	1.4	75*	475	air	7	4.301 (2)

* Flux was 55 mol% KOH/45 mol% KCl.

were measured using a SQUID. For such a sample, $T_c = 30.5$ K and the transition is sharp (Fig. 1), indicating good sample homogeneity. (Some broadening occurs because the measuring field of 25 Oe is a substantial fraction of the lower critical field, H_{c1} , which is ~ 110 Oe at 2 K (ref. 3).) The sample used for Fig. 1 was grown in air; surprisingly, transitions for samples grown in an O_2 atmosphere had similar superconducting onset temperatures but transitions were much broader for similar melt compositions. In contrast, annealing in oxygen was necessary to obtain the highest T_c s in ceramic $\text{Ba}_{1-x}\text{K}_x\text{BiO}_3$ samples. The broadening for single-crystal samples grown in oxygen is possibly due to a less uniform K distribution or a preference for a higher K concentration in crystals grown in more oxidizing conditions.

Although the single crystals investigated often had large mosaic spreads, crystals suitable for single-crystal X-ray analysis could be obtained by cleaving until a single or bidomain crystal was obtained. Structure determinations were performed for crystals containing 4 and 40 mol% potassium. Integrated intensities up to $2\theta = 100^\circ$ were measured using an ENRAF-NONIUS CAD4 single-crystal diffractometer and were corrected for absorption. Calculations were carried out using the NRCVAX structure package¹⁴. Atomic positions and anisotropic temperature parameters of $\text{Ba}_{0.96}\text{K}_{0.04}\text{BiO}_3$ and $\text{Ba}_{0.626}\text{K}_{0.374}\text{BiO}_3$ are given in Table 2. The lattice parameters were determined by measuring absolute 2θ values of at least 28 reflections with $2\theta = 50$ – 65° . The unit cell observed for orthorhombic $\text{Ba}_{0.96}\text{K}_{0.04}\text{BiO}_3$ is four times larger than that of the simple perovskite cell as a result of a small structural distortion. In both structures, the oxygen anisotropic thermal parameters indicate a larger mean square displacement perpendicular to the Bi–O–Bi direction. This is common to perovskites and reflects

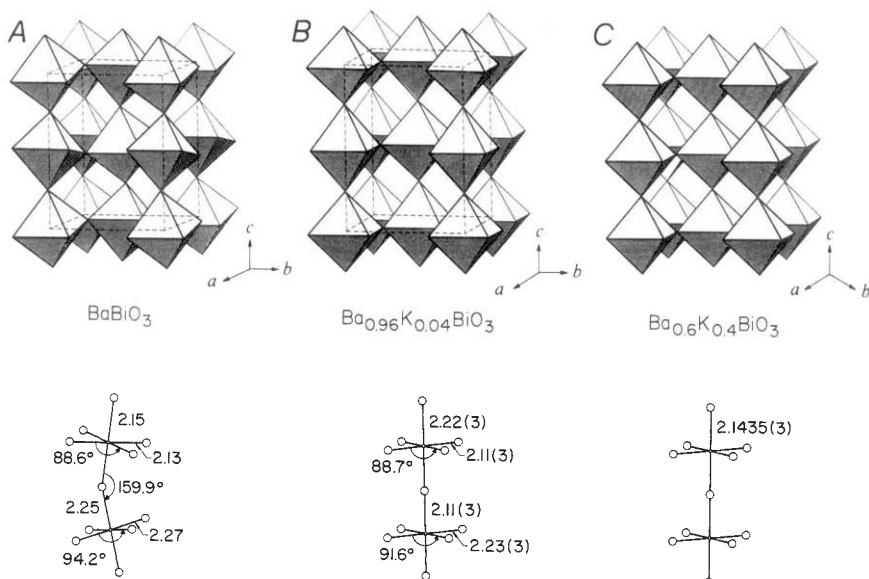


Fig. 2 Structural representations of $\text{Ba}_{1-x}\text{K}_x\text{BiO}_3$ phases for A, $x=0$, B, $x=0.04$ and C, $x=0.4$. Dotted lines indicate the unit cell. Lower portions of the figure give key bond distances and angles.

Table 2 Crystallographic data and anisotropic thermal parameters

Atom	Site	x	y	z	B_{iso} ($\text{\AA}^2$)	Occupancy	u_{11}	u_{22}	u_{33}	u_{12}
Ba_{0.96}K_{0.04}BiO₃*										
K	4j	0	$\frac{1}{2}$	0.25295 (17)	1.11 (4)	0.04 (1)	1.63 (6)	1.36 (5)	1.23 (5)	-3.0 (15)
Ba	4j	0	$\frac{1}{2}$	0.25295 (17)	1.11 (4), 0.96 (1)					
Bi 1	2a	0	0	0	0.595 (25)					
Bi 2	2c	$\frac{1}{2}$	$\frac{1}{2}$	0	0.77 (3)					
O 1	8n	0.260 (6)	0.254 (4)	0	5.6 (16)					
O 2	4i	0	0	0.244 (4)	7.3 (34)		12.4 (50)	14.5 (56)	0.9 (10)	
Ba_{0.626}K_{0.374}BiO₃†										
K	1a	0	0	0	0.81 (3)	0.374 (8)	1.02 (4)	1.02	1.02	
Ba	1a	0	0	0	0.81 (3)	0.626 (8)				
Bi	1b	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	0.412 (11)					
O	3c	$\frac{1}{2}$	$\frac{1}{2}$	0	1.79 (22)					

Anisotropic thermal parameters in $100 \times \text{\AA}^2$.

* Orthorhombic lattice, space group *Immm* (no 71). $a = 6.1298$ (5) $\text{\AA}$, $b = 6.1374$ (5) $\text{\AA}$, $c = 8.6628$ (7) $\text{\AA}$, $Z = 4$; 1340 reflections measured, 775 unique, 466 observed ($I > 2\sigma(I)$), $R_F = 0.062$.

† Cubic lattice, space group *Pm3m* (no 221), $a = 4.2869$ (6) $\text{\AA}$, $Z = 1$; 676 reflections measured, 112 unique, $R_F = 0.022$.

the inherent tendency of the metal-oxygen-metal bonds to buckle.

The structure of BaBiO₃ is shown in Fig. 2A (refs 5-7). This material has a monoclinic distorted perovskite structure at room temperature. Octahedra of two sizes form a staggered arrangement with the larger having Bi-O distances of 2.25-2.27 $\text{\AA}$, and the smaller, of 2.13-2.15 $\text{\AA}$. The inequivalent Bi-O arrangements can be analysed in terms of bond-length/bond-strength relationships (for numerical parameters, see ref. 7), indicating a pronounced charge disproportionation, formally $\text{Bi}^{4+} \rightarrow \text{Bi}^{3.5+} + \text{Bi}^{4.5+}$.

The structure of a crystal from batch 8 (Table 1), which showed a T_c of 30.5 K, was refined as cubic, space group *Pm3m*, with $a = 4.2870$ (3) $\text{\AA}$. The potassium stoichiometry was determined by refinement, constraining the A site to full occupancy by Ba and K, and yielded the composition Ba_{0.626}K_{0.374}BiO₃. The BiO₆ units are equivalent and regular with a Bi-O distance of 2.144 $\text{\AA}$ (Fig. 2C). The Ba(K) coordination polyhedron is the regular cubo-octahedron with a uniform Ba(K)-O distance of 3.03 $\text{\AA}$. Ordering of the Ba and K atoms was not observed. Isotropic thermal parameters of Bi and Ba(K) agree well with previously reported values^{5,6}. The anisotropically refined oxygen thermal parameters of superconducting Ba_{0.6}K_{0.4}BiO₃, like those of BaBiO₃, have values typical of perovskites.

The structure of Ba_{0.96}K_{0.04}BiO₃ (Fig. 2B) is derived from BaBiO₃ by aligning the BiO₆ octahedra along the *c*-axis. The symmetry is orthorhombic, space group *Immm*, with $a = 6.1298$ (5) $\text{\AA}$, $b = 6.1374$ (5) $\text{\AA}$ and $c = 8.6628$ (7) $\text{\AA}$. Although cell parameters are close to those observed for BaBiO₃, bond distances have changed significantly. Two types of Bi sites remain: the structure is composed of alternate uniaxially elongated and compressed BiO₆ octahedra, one with four short (2.11 $\text{\AA}$) and two long (2.22 $\text{\AA}$) Bi-O distances, and the other with four long (2.23 $\text{\AA}$) and two short (2.11 $\text{\AA}$) Bi-O distances. As in BaBiO₃, the inequivalent Bi-O sites reflect a disproportionation of the bismuth charge, but the magnitude of the charge difference is reduced from ~ 1 in BaBiO₃ to ~ 0.3 . This structure can be regarded as frozen in a soft mode of the perovskite structure, resulting in a commensurate charge density wave and a displacive modulation of the oxygen positions. The 6% *R* factor reflects the large mosaic spread, but indicates that the structure is well modelled. We cannot, however, rule out the possibility of a subtle reduction in symmetry or of a superlattice. In fact, the oxygen thermal parameters are larger than those for Ba_{0.6}K_{0.4}BiO₃, indicating apparent motion of the oxygen atom perpendicular to the Bi-O-Bi axis, which is possibly due to (small) incoherent static rotations of the BiO₆ octahedra similar to those in BaBiO₃. Refinement of such models did not, however, lead to substantial improvement.

Preparation by ceramic techniques of single-phase Ba_{1-x}K_xBiO₃ with superconducting T_c near 30 K requires synthesis under oxygen pressure¹ or nitrogen followed by an oxygen anneal⁸. The direct crystal growth of Ba_{1-x}K_xBiO₃ from KOH fluxes in air without the need for oxygen annealing is remarkable. Here, superconductivity was observed only for $a < 4.3$ $\text{\AA}$, in agreement with studies on polycrystalline samples^{1,8}. Our results and those of Hinks *et al.*⁸ differ in the Ba:K ratio necessary for superconductivity, thus necessitating further study of the proposed T_c versus *x* phase diagram⁸. In these studies the K stoichiometry (*x*) is refined from single crystal X-ray and neutron powder data, respectively. Determining the ratio from refinement alone can be misleading, but attempts to use energy-dispersive and Auger spectroscopy on single crystals have been inconclusive. The neutron study on a nominal $x = 0.4$ sample gave $x = 0.29$ in a model with only Ba and K on the A site, but $x = 0.35$ if Bi (3%) was also allowed on the A site⁸. In general, different analytical techniques give consistent lattice parameters and T_c s for Ba_{1-x}K_xBiO₃ samples but are less unanimous regarding the composition. Table 1 reveals the possibility that K-Ba miscibility gaps may occur under single-crystal growth conditions, reminiscent of BaPb_{1-x}Bi_xO₃ (ref. 15).

No superconductivity is observed in Ba_{1-x}K_xBiO₃ for low *x*. We have determined that the commensurate supercell for low *x* involves oxygen modulation and is significantly different from that of BaBiO₃. The very small metric distortion at $x = 0.04$ and the cubic cell found earlier for $x = 0.13$ (ref. 9) suggest that the cubic phase first appears near $x \approx 0.10$, although Hinks *et al.*⁸ reported an orthorhombic cell for $x < 0.25$. Metallic behaviour and the possibility of superconductivity are favoured by equivalence of Bi sites. This is the situation at $x = 0.374$, for which the structure is undistorted and $T_c = 30.5$ K.

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Fracture in microsphere monolayers studied by experiment and computer simulation

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The formation of irregular (often fractal¹) patterns under non-equilibrium conditions has become a subject of considerable scientific and practical interest. One of the most important processes of this type is the formation of cracks and other extended defects in materials under stress^{2,3}. Here we present an experimental study of crack growth in a two-dimensional system, using a monolayer of uniformly sized microspheres confined between two parallel sheets of glass. The cracking patterns observed in this system closely resemble those found in more complex systems of practical importance, such as paint films or ceramic-coated metals. A simple two-dimensional computer model for elastic fracture leads to structures that closely resemble those observed in the experiments. In both the experimental and computer models an early stage in which isolated defects are formed is followed by a period in which rapid growth of almost linear cracks occurs. At later times the crack growth process slows down and the shapes of the cracks become increasingly irregular.

The two-dimensional material used in our experiments was prepared from uniformly sized spheres of sulphonated polystyrene with an effective diameter d_1 of $3.4 \mu\text{m}$ ($\pm 1\%$) dispersed in water⁴. By confining the spheres between parallel planar glass sheets it was possible to form a polycrystalline monolayer with a relatively large grain size (typically 10^5 – 10^6 spheres). A strained film was produced by allowing the monolayer to slowly dry. During this drying process the sphere diameter is reduced to $d_0 = 2.7 \mu\text{m}$.

A variety of different features was observed in the strained film. The first cracks were formed along the grain boundaries and had a tendency to pass through lattice defects such as vacancies and impurities. But here we are concerned primarily with crack propagation inside regular grains with relatively few defects. At the earliest stage in the cracking process, small (localized) defects are formed at a relatively slow rate. This stage is followed by the fast propagation of more-or-less linear cracks (Fig. 1a). In the later stages the crack growth process becomes slower and the cracks become progressively less linear (Fig. 2a).

Results from a simple two-dimensional model which reproduces these characteristics are shown in Figs 1b and 2b. This model is based on a model for elastic fracture in thin films⁵ in which the close-packed monolayer of microspheres is represented by a triangular network of nodes and bonds that initially form a triangular lattice (Fig. 3). Each node is connected to six nearest neighbours by bonds with an equilibrium bond length of l_0 (equivalent to the microsphere diameter, d_0 , in the dried monolayer). We assume that the energy associated with this two-dimensional network is given by a harmonic approximation:

$$E_1 = \frac{1}{2} k_1 \sum_i (l_i - l_0)^2 \quad (1)$$

where l_i is the length of the i th bond and k_1 is the bond force constant.

In addition, each of the nodes is assumed to be bonded to an underlying rigid substrate by a relatively weak bond. The energy associated with this bond is given by

$$E_2 = \frac{1}{2} k_2 \sum_j |\mathbf{r}_j - \mathbf{r}'_j|^2 \quad (2)$$

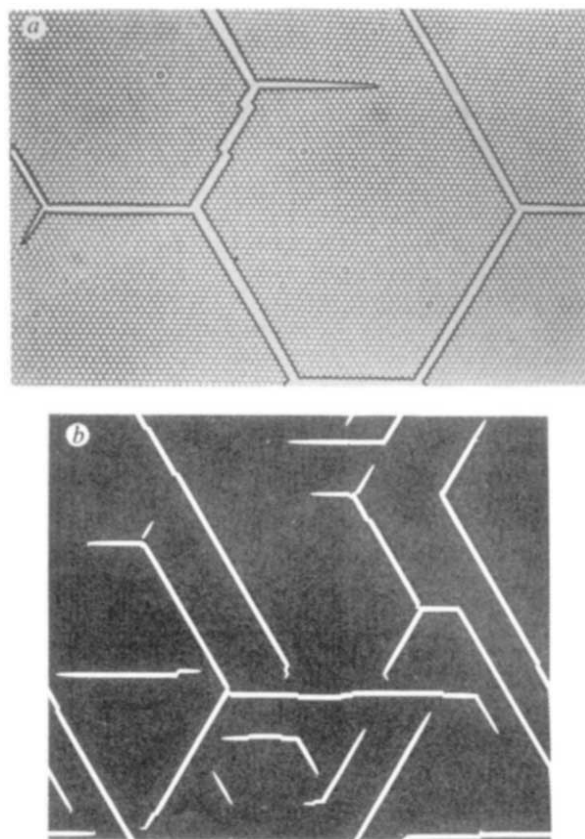


Fig. 1 An early stage in the crack propagation process. *a*, Optical micrograph of a part of a single grain in a much larger system. *b*, Simulation carried out using 40,000 nodes and 120,000 bonds with the parameters $k_1 = 400$, $k_2 = 40$ and $\delta_{\max} = 0.25$. The initial strain ($\sigma = l_1/l_0$) was 1.2, which corresponds quite closely to that used in the experiment ($d_1 = 3.4 \mu\text{m}$, $d_0 = 2.7 \mu\text{m}$, $d_1/d_0 = 1.26$); 3,000 bonds have been broken at the stage shown in this figure.

Here $\mathbf{r}_j$ is the position of the j th node and $\mathbf{r}'_j$ is the position of attachment to the substrate (denoted by X in Fig. 3).

At the start of each simulation the network is stretched isotropically so that each bond in the triangular network has a length l_1 (equivalent to the diameter d_1 in the experiments). Periodic boundary conditions are used in these simulations so that every node is joined to six nearest neighbours. As the simulation proceeds, bonds in the hexagonal network are selected at random and broken with a probability given by

$$P_i \propto e^{\frac{1}{2} k_1 (l_i - l_0)^2} \quad (3)$$

These probabilities are normalized so that the largest bond-breaking probability associated with any of the bonds is 1.0. After a bond has been broken, the system is relaxed to a new mechanical equilibrium (the energy $E_1 + E_2$ is minimized) and the process is repeated. If, during the mechanical relaxation process, the distance $|\mathbf{r}_j - \mathbf{r}'_j|$ between a node and its point of attachment to the underlying substrate exceeds a value of $\delta_{\max}$, then the point of attachment, X, is moved towards the current position of the node until $|\mathbf{r}_j - \mathbf{r}'_j|$ becomes equal to $\delta_{\max}$. To avoid 'overshoots' in the movement of the positions of attachment to the substrate, over-relaxation was not used in these simulations. The process of bond-breaking and relaxation is repeated many times to simulate the crack propagation process. These simulations can easily be made time-dependent by incrementing the time by an amount $1/(P_{\max} N)$ each time a bond is selected at random (whether or not it is actually broken). Here N is the total number of bonds and $P_{\max}$ is the maximum value of $\exp[(k_1/2)(l_i - l_0)^2]$ for any bond in the system.

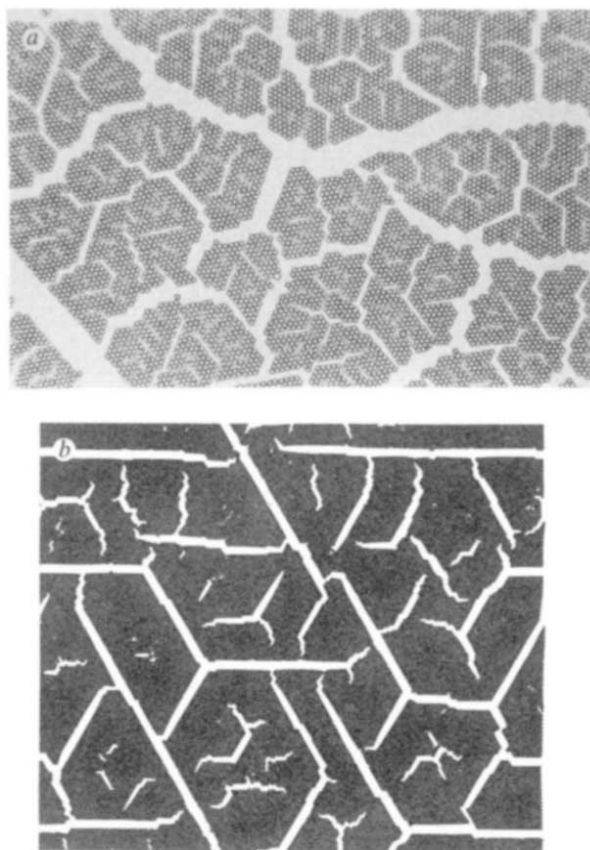


Fig. 2 A relatively late stage in the crack propagation process. *a*, Micrograph obtained from the same experiment as in Fig. 1*a*. *b*, Simulation with $k_1 = 300$, $k_2 = 30$, $\delta_{\max} = 0.15$, $\sigma = 1.20$. 7,500 bonds have been broken.

This model is based on the idea that the polymer microspheres are relatively strongly bonded to each other and weakly bonded to the glass surfaces which confine the microspheres to a monolayer. In the model we consider attachment to only one surface but this is equivalent to attachment to both surfaces with bonds which have a force constant of $\frac{1}{2} k_2$. We also assume that the bond-breaking probabilities (crack propagation rates) are very sensitive to the local stress field (as indicated by equation (3)). But the qualitative aspects of our results are not sensitive to the precise form of the relationship between P_i and $(l_i - l_0)$ provided that P_i increases sufficiently rapidly with $(l_i - l_0)$ (ref. 5).

Both the experimental and simulation results are consistent with the following scenario. At the early stages, after the monolayer has dried, the stress is high but the stress concentrations are low. Under these conditions isolated defects are formed relatively slowly. Eventually, some of the isolated defects grow and a large stress concentration builds up at the crack tips. (Alternatively a crack can be induced externally.) Because of the large stress concentration at the ends of the cracks at this stage, they grow very rapidly and the ensuing cracks are almost linear. This initial cracking reduces the average stress in the system, so that subsequent cracking becomes progressively less rapid and the shapes of the cracks increasingly irregular. If there were no bonding between the monolayer and the substrate(s), the stress would be completely relieved after the first few cracks had appeared and no further cracking would occur. Figure 4 shows the fracture pattern at the end of an experiment (at four different magnifications). At the smallest magnification about 500,000 microspheres are shown (compared to the 40,000 nodes

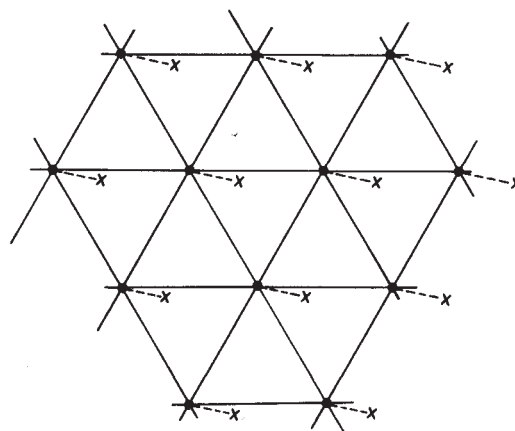


Fig. 3 A representation of the model used in this work. The nodes (large dots) are connected by strong bonds to form a triangular lattice. Each of the nodes is joined to an underlying rigid substrate at X by a weak bond (dashed line). The motion of the nodes is confined to a plane parallel to the substrate. This figure shows the configuration at the start of a simulation.

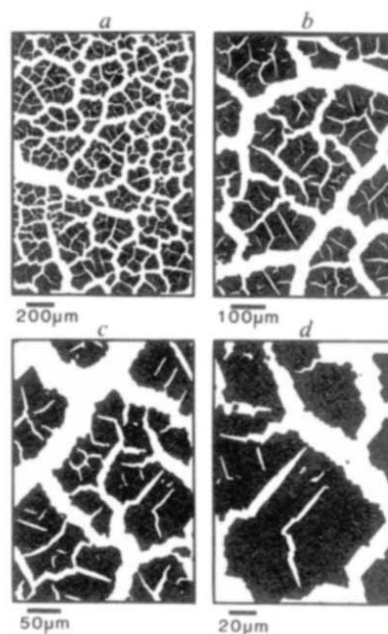


Fig. 4 The final fracture pattern obtained using a monolayer of 3.4- μm spheres. Four different magnifications are shown.

used in the simulations), although the experimental system contained a total of $\sim 4 \times 10^7$ spheres.

It has been shown recently that mechanical fracture (cracking) processes in real materials^{6,7} may lead to the generation of fractal surfaces. In addition, the results from simple two-dimensional computer models^{8,9} also suggest that cracks formed under a variety of conditions may have a fractal geometry. It is now well established that fractal structures are generated by closely related processes such as dielectric breakdown¹⁰ and fluid-fluid displacement processes^{11,12}, which can be described in terms of the diffusion-limited aggregation (DLA) model¹³. In these processes and in the crack growth model of Louis and Guinea⁸, the pattern growth is a non-local process which is controlled by a field which obeys either the Laplace equation¹⁴ (for DLA) or the Navier equation¹⁵ (for crack growth). In our case the weak attachment between the two-dimensional medium and the substrate is sufficient to localize the effects of both isolated and

extended defects⁵. Consequently, the crack-growth process explored here is not closely related to DLA. Nevertheless, the results shown in Fig. 4 do suggest that the cracking patterns might be described in terms of the concepts of fractal geometry¹. A preliminary investigation (A.T.S., unpublished work), in which the box-counting method¹⁶ was used to analyse digitized representations of Fig. 4 (and similar micrographs), gave an effective fractal dimensionality (D) of 1.68 ± 0.06 . That this is quite close to the value found from two-dimensional DLA simulations ($D = 1.71$) (ref. 17) is coincidental.

The cracking patterns generated by these simple experimental and computer models resemble quite closely those found in a variety of more complex systems such as paint films and thin film deposits. We believe that the study of simple model systems can provide valuable insights into the failure of more complex systems which are often of considerable practical importance.

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Table 1 Open/close schedule of the sediment trap and particle fluxes

Sample number	Duration (days)	Particle flux ($\text{mg m}^{-2} \text{d}^{-1}$)	Days	Calendar date
Open 1	11	0.62	25	25 Jan. 1985
2	11	1.43	36	5 Feb.
3	11	2.92	47	16 Feb.
4	11	3.79	58	27 Feb.
5	11	9.20	69	10 Mar.
6	11	4.66	80	21 Mar.
7	22	0.37	91	1 Apr.
8	22	0.06	113	23 Apr.
9	22	0.02	135	15 May
10	22	0.005	157	6 June
11	22	<0.001	179	28 June
12	22	<0.001	210	20 July
13	22	<0.001	223	11 Aug.
14	22	<0.001	245	2 Sept.
15	22	<0.001	267	24 Sept.
16	22	<0.001	289	16 Oct.
17	22	<0.001	311	7 Nov.
18	22	<0.001	333	29 Nov.
19	22	<0.001	355	21 Dec.
20	11	0.02	377	12 Jan. 1986
21	11	0.11	388	23 Jan.
22	11	0.33	399	3 Feb.
23	11	1.06	410	14 Feb.
24	11	0.30	421	25 Feb.
25	11	0.34	432	8 Mar.
Close			443	19 Mar.

The trap was deployed at the northern Weddell Sea station, $62^{\circ}26.5' \text{ S}$, $34^{\circ}45.5' \text{ W}$, 863-m water depth. Opening/closing of the trap as controlled by a microprocessor-based timer and the operation was verified by an independent computer after recovery. All opening/closing times were 12:00 noon GMT.

Mk 5-25 time-series sediment trap³ at 863 m depth from January 1985 to March 1986; this trap has a 1.2-m^2 opening and is capable of collecting 25 sequential time-specific samples. Based on our results from experiments at the Bransfield Strait⁴ we programmed sampling periods of different trapping durations: six periods, each of 11-day duration, were set from 25 January to 1 April 1985; these were followed by 13 periods, of 22-day duration, from 1 April 1985 to 12 January 1986, and finally, another six periods, of 11-day duration, from 12 January to 19 March 1986.

The dry mass flux was collected on preweighed, gridded Nuclepore filters. The amounts of biogenic silica (opal), carbonate and lithogenic particles (clay) were estimated from the Si, Al and Ti content, which were determined by inductively coupled plasma spectrometry analysis (ICPS)⁵. Content of combustible organic matter was estimated as the difference between the total flux and the sum of the opal, carbonate and lithogenic components. These values agreed with results from organic elemental analysis⁶.

The annual particle flux measured at the northern Weddell Sea station from 25 January 1985 to 23 January 1986 was 371 mg m^{-2} , with biogenic opal, combustible matter, carbonate and the clay particles making up 79, 17, 3 and 1% of the total flux, respectively. Biogenic material comprised 99% of the total flux. The seasonal variability in the daily flux was very high (Fig. 1), ranging from a maximum flux of 9.2 mg m^{-2} per day during March 1985 to almost zero for July 1985 to January 1986.

Significant particle flux occurred from the start of the experiment, when sea ice retreated southwards of the mooring site. The flux increased rapidly and reached its maximum about 10 weeks after the ice opened, at which time the ice edge had retreated about 950 km to the south of the station (Fig. 1). Ice around the mooring site loosened at the beginning of 1986, and the ice edge passed the station at the end of January 1986. During the 1986 study period, the flux maximum occurred between February 14 and 25 (sample 23), about six weeks earlier

Seasonal variability of particle flux in the Weddell Sea and its relation to ice cover

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In the Weddell Sea, primary production varies seasonally as a result of the solar cycle and the large-scale oscillation of the ice edge across much of its area. The annual ice transgression is the largest of any region on Earth¹ and has a profound influence on the production and transportation of particulate matter. In order to clarify the flux, origin and mode of vertical transport of oceanic particles in the pelagic Weddell Sea, we deployed a multi-year sediment trap. The annual particle flux measured was the smallest yet observed in the world ocean, and showed extreme variability. Phytoplankton production is at least partly seeded by diatoms released from the melting of sea ice which had formed in the coastal area of the Antarctic continent. Phytoplankton production under the winter pack ice appears to be minimal.

The year-round mooring station WS-1 is located at $62^{\circ}26.5' \text{ S}$, $34^{\circ}45.5' \text{ W}$, in a water depth of 3,880 m. This station is covered by sea ice for about 70% of the year (≥ 9 on ice-cover scale (ICS); see refs 1 and 2). We developed an automated Parflux

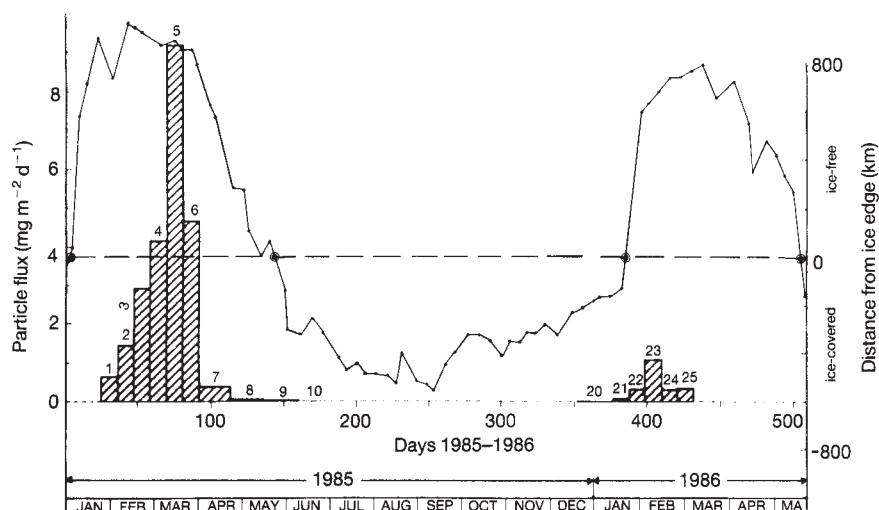


Fig. 1 Total flux for each period (in $\text{mg m}^{-2} \text{d}^{-1}$, shaded bar graph). Sample numbers are on top of the bars. Superimposed is the shortest distance (km) from the approximate ice edge to the sediment-trap site three weeks before, during, and two months after the experiment, based upon weekly Antarctic Ice Charts, NPOC, which were compiled from the NOAA polar orbiter, NASA Nimbus 7 SMMR, GEOSTAT altimeter, and visual data, using NOPC ice-coverage scales.

than the 1985 peak. Its value was only 12% of the 1985 maximum. During this period, in both 1985 and 1986, the ice edge was 600–700 km south of the station.

As observed at other southern ocean stations^{4,7,8}, fecal pellets played an important role in the vertical transport of material. Pellets were ~0.5 mm in diameter when wet, and were slightly elongated. Cylindrical-shaped pellets of ~200 μm length and ~50 μm width, which are produced by krill, were rarely found. Diatom frustules were the major constituents of fecal pellets. The total mass flux correlates well with the mass flux of fecal pellets (correlation coefficient $R = 0.90$). An annual pellet flux of about $94,000 \text{ m}^{-2}$ and an average pellet dry weight of ~4 μg was estimated.

We suggest that the variability of particle fluxes at this station is due to spring thaw processes rather than to the availability of larger open seas, because (1) the abrupt increase in particle flux followed the opening of the ice, and (2) the sudden and near-complete termination of particle sedimentation occurred while the ocean was still open. Although loosening of sea ice generally began in late November and the ice broke to 5–7 ICS early in the following year, the flux did not increase at that time but only after the near-complete melting of local sea ice. Melting resulted in the release of in-ice particulate organic carbon (POC) into the water column^{9,10}.

The maximum fluxes of siliceous biogenic particles occurred during the periods of highest total flux. In general, more than 95% of the siliceous particles are diatom valves. Silicoflagellates (*Distephanus speculum* group) provide up to 15% (samples 1, 8, 9 and 21) while radiolarians and chrysophycean cysts contribute only minor amounts. Average flux values of diatom valves are $\sim 10^6$ – $10^7 \text{ m}^{-2} \text{d}^{-1}$ during periods of high flux. Diatom species compositions and relative abundances are more or less uniform in all samples containing large amounts of siliceous particles. About 40 diatom species were identified. Most abundant is *Nitzschia curta* (35% of the total diatom assemblage),

followed by *Thalassiosira gracilis* (30%), *Nitzschia cylindrus* (15%) and *Nitzschia lineola* (5%). Both *Nitzschia curta* and *Nitzschia cylindrus*, known as neritic species, are restricted to the near-continent environment and are related to the presence of sea ice¹¹. Gersonde¹² reports high abundances for both species in new frazil slush ice produced during the austral fall and in cores of one-year-old ice recovered from the coastal area of the Weddell Sea south of the WS-1 mooring position. *Nitzschia kerguelensis*, which is the most prominent 'oceanic' species in the Circumantarctic Ocean¹¹, contributes only minor amounts to the total assemblage. This fact supports the theory that summer phytoplankton-production in the northern Weddell Sea is at least partly seeded by diatoms released from melting sea ice. This sea ice is produced in the coastal area of the Antarctic continent and is transported northwards by the cyclonic Weddell Gyre. Seeding of phytoplankton blooms by algae released from sea ice has also been reported for the Ross Sea¹³.

We saw no evidence of higher zooplankton-mortality at the end of the high-flux season (this would be manifested, for example, in an increase of carcasses). We propose migration of the grazer population in the direction of the ice edge to the south¹⁴ as an explanation for the low flux during period 7 and possibly periods 24 and 25. The small flux of fecal pellets throughout the ice-packed winter season suggests that a small population of zooplankton was feeding directly on bottom-ice algae⁹.

Carbonate particles were a minor constituent of the flux throughout the year. The flux of captured exoskeletons of dwarfed planktonic foraminifera was 0.1–2 per m^2 per day. Small grains of quartz and feldspar, <10 μm in diameter, were seen rarely under the scanning electron microscope. Wide-area scanning energy dispersive X-ray spectrometry of fecal pellets occasionally showed a signal for Al, indicating that the zooplankton ingested clay particles.

An explanation is required for the much smaller particle flux

Table 2 Particle flux from the northern Weddell Sea compared to other data

	Weddell Sea 62°26' S 34°45' W Jan. 1985–Dec. 1985 $\text{mg m}^{-2} \text{yr}^{-1}$	Bransfield Strait 62°15' S 57°31' W Dec. 1983–Nov. 1984 $\text{g m}^{-2} \text{yr}^{-1}$	Greenland Basin 74°35' N 06°43' W Aug. 1985–Jul. 1986 $\text{g m}^{-2} \text{yr}^{-1}$	C. Fram Strait 78°52' N 01°22' E Aug. 1984–Aug. 1985 $\text{g m}^{-2} \text{yr}^{-1}$	Station P 50°00' N 144°59' W Nov. 1985–Oct. 1986 $\text{g m}^{-2} \text{yr}^{-1}$	Panama Basin 5°22' N 85°35' W Dec. 1979–Dec. 1980 $\text{g m}^{-2} \text{yr}^{-1}$
Total	371	107.7	10.8	6.6	45	93.3
Biogenic	367	53.7	7.1	2.9	44.7	65.1
Carbonate	11	5.2	3.3	1.4	21.9	43.7
Opal	293	38.8	2.6	0.6	19	10.9
Combustible	63	9.7	1.2	0.9	3.8	10.5
Lithogenic	4	53.5	3.1	4	0.3	28.2
POC	20	3	0.4	0.4	1.1	3.7

The data were obtained during roughly the same time period (except for Panama Basin data) using one-year time-series experiments from the Arctic, Northern Pacific, Antarctica and a hemipelagic station. Note that the flux units for the Weddell Sea station are mg m^{-2} and g m^{-2} for all other stations.

collected during the 1986 austral summer relative to the 1985 summer. We offer the following hypotheses: (1) this experiment would have missed any major particle-flux events occurring after 19 March 1986, when the trap was closed, and/or (2) the 1985 spring thaw released sea ice over a wide area (as indicated by satellite images), thereby releasing much in-ice POC into the water column. This would stimulate the rapid growth and/or immigration of the zooplankton community at the trap site. We note in this respect that satellite images in early 1986 show that the ice edge retreating over the site had a sharp boundary line with the open ocean, thus releasing in-ice POC over a larger period and at a slower rate than in 1985.

The mode of particle flux observed in the northern Weddell Sea in 1985 and part of 1986 was considerably different from that in the Greenland Sea (Table 2), despite the similarity of annual ice-edge oscillations in both areas: the annual biogenic particle flux observed at a Greenland Sea station was about 20 times greater than that from the northern Weddell Sea station. The contents differed significantly in that the Greenland Sea biogenic flux consisted of about 26% carbonate (coccospheres, coccoliths and planktonic foraminifera) and 37% opal particles, while the northern Weddell Sea biogenic flux consisted mostly of opal frustules. The organic N to organic C ratios in the two areas were comparable, with values of 6.6–7.7. The atomic ratio of organic C to carbonate C was about one in the Greenland Sea, while it ranged as high as 15.5 at the northern Weddell Sea station. The ratio of organic C to Si in biogenic opal was 0.34 in the Greenland Sea and 0.16 in the Weddell Sea.

Annual biogenic and lithogenic particle fluxes measured at the Weddell Sea station had the lowest values yet observed in the ocean. The primary production in the open-ice water column in the north-central Weddell Sea was related to blooms at the marginal ice zone^{15,16}. Jennings *et al.*¹⁵ used seasonal nutrient depletions in the eastern Weddell Sea for calculations of the average primary production, which they estimate at 220–420 (mg carbon) m⁻² d⁻¹ in the springtime. Assuming the lower value for a 90-day period results in a minimum production of 20 (g carbon) m⁻². This is three orders of magnitude higher than our 1985 flux. Such a discrepancy is unusually high compared to other oceans¹⁷ and may be due to a high degree of spatial and temporal variability of primary production in the Weddell Sea. El-Sayed and Taguchi¹⁸ demonstrated that oceanic regions support relatively low production whereas coastal waters support elevated production.

Estimates derived from a local sedimentation rate of about 1.4 cm per 1,000 yr (ref. 19; Müller and Wefer, personal communication) give annual accumulation rates of 40 mg m⁻² for organic carbon and 300 mg m⁻² for opal. A very low annual organic-carbon accumulation-rate of 40 mg m⁻² has also been calculated by Müller and Wefer (personal communication) for a core in the north-western Weddell Sea. They estimate that only 0.02–0.07% of the carbon produced in surface waters becomes preserved in the sediments of this region.

Within the limits of this experiment, the input of new organic carbon and new nitrogen into the northern Weddell Sea appears to make an insignificant contribution to the world ocean primary production. Although the percentage of silica in the trapped sediment was large, the annual flux of about 0.18 g m⁻² was small compared to the 2.6 g m⁻² measured in the Greenland Sea. This also indicates that the northern Weddell Sea is not a major source of dissolved silica for Antarctic bottom-water. However, the Weddell Sea shelf region may still represent a large source-area of silica²⁰.

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Simple magma supply geometry inferred beneath a segment of the Mid-Atlantic Ridge

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The petrological characteristics of axial basalts along the global mid-ocean ridge system shed light on the processes of magma supply involved in the formation of ocean crust. Several models of magma supply^{1–4} predict regular patterns of chemical variation along the strike of mid-ocean ridge axes. Here we present chemical data from closely spaced samples of axial basalts from the Mid-Atlantic Ridge, between the Rio Grande and Moore fracture zones at ~26° S. This segment of the ridge is far from active hotspots and erupts normal mid-ocean-ridge basalt. The samples show regular patterns of chemical variation along the ridge axis, indicating systematic and correlated differences in depth and extent of partial melting and extent of shallow fractionation. These patterns support the existence of a deep central supply system but preclude along-axis melt migration and the presence of large well-mixed chambers below this segment of the southern Mid-Atlantic Ridge.

The petrological characteristics of basalts erupted along the axis of mid-ocean ridges (MORs) can help to constrain models of sub-axial mantle flow, thermal structure, melting processes and magma supply^{3–13}. To investigate magma supply processes at slow spreading rates, we sampled a 100-km-long segment of the Mid-Atlantic Ridge (MAR) at 26° S. This segment has a low spreading rate (35–40 mm yr⁻¹)^{14,15} and offers an ideal locale for distinguishing between central^{1–4} and multiple¹⁶ magma supply models because of the simple along-axis profile shape and the absence of hotspot influence¹⁷. In the 26° S area, the MAR is offset by the Rio Grande and Moore fracture zones (Fig. 1). This ridge segment has a deep median rift valley along most of its length but in the centre has an axial high with no rift valley. Figure 1 shows dredge locations on the axis, slightly off the axis and on ridge-flank seamounts. The morphology, abundance and distribution of these seamounts is discussed separately¹⁸.

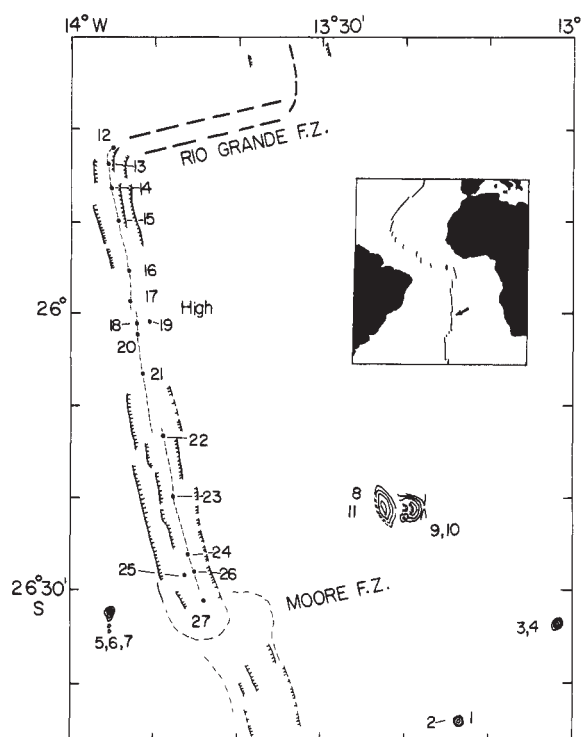


Fig. 1 The Mid-Atlantic Ridge between the Rio-Grande and Moore fracture zones, showing dredges along the MAR axis (D12-18, 20-24, 26, 27), slightly off-axis (D19 and 25) and on ridge-flank seamounts (D1-11).

The dredges were navigated using Seabeam and are very well located. All the axial dredges recovered extremely fresh glassy basalt. Table 1 presents several representative major-element analyses. In all but two cases, all the specimens recovered in each dredge are similar in appearance and chemically identical within experimental error. We thus infer that in most cases we sampled only a single basalt flow. In dredges 21 and 22, the observed chemical variability can be ascribed to shallow-level fractional crystallization, which possibly occurred during shallow ascent and/or eruption, as inferred for basalts from the Kane fracture zone¹⁹ and other sets of mid-ocean-ridge basalt (MORB).

The analysed samples (total of 126 analyses) are all depleted MORB. They do not display the simple orderly chemical variations, resulting from shallow fractionation processes, that are typically exhibited by suites of MORB^{5-8,19}. Binary plots of major oxide against MgO content (Fig. 2) show little or no correlation. With the exception of the intra-dredge variation in D21 and D22, the samples cannot be related to each other by shallow differentiation from a single parent or by simple mixing processes.

In contrast to the poor correlations on MgO variation diagrams, the samples display more systematic chemical and mineralogical variation patterns spatially, along the spreading axis (Fig. 3). For example, the northernmost four axial samples show fairly regular decreases in Mg#, Na_{8.0} and Fe_{8.0} (where Mg# = 100 × Mg/(Mg + Fe²⁺), and Na_{8.0} and Fe_{8.0} are the sodium and iron contents that the sample would have at 8 wt% MgO; see Fig. 2 legend.) Samples from these dredges (D12-15) cannot be related by shallow fractionation, because with falling MgO content, TiO₂ decreases and CaO increases monotonically (Fig. 2), opposite to the trends expected. A similar, although less regular pattern is observed near the Moore fracture zone. The centre of the segment, like the ends, has relatively high

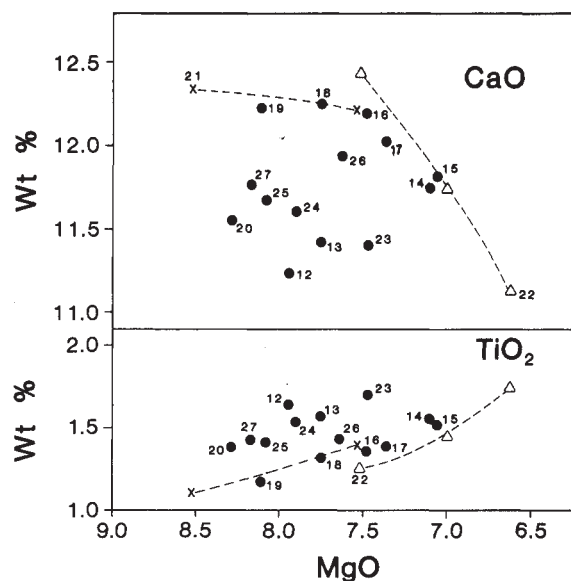


Fig. 2 Plots of CaO and TiO₂ contents against MgO content for sample averages of chemically homogeneous groups in each dredge. Except for D21 (crosses) and D22 (triangles) the samples in each dredge are chemically identical within experimental error. Samples from D21 and D22 can be related by fractional crystallization; most others cannot. Note the absence of the single linear trend usually displayed by suites of MORB. Plots of other oxides, such as FeO and Na₂O, display similar scatter, implying that most samples were derived from chemically distinct parents.

Mg#, Na_{8.0} and Fe_{8.0}, and values in between these regions vary to form W-shaped patterns as functions of latitude (Fig. 3). Although not shown, Al₂O₃, K₂O and P₂O₅ analyses also show W patterns, whereas SiO₂, CaO and CaO/Al₂O₃ exhibit inverted W patterns. By comparison with experimental results (summarized in ref. 20), these major-element variation patterns are of the form expected to arise from differences in the extent and depth of melting of a homogeneous mantle peridotite beneath the axis.

Differences in Na_{8.0} are interpreted as reflecting differences in the extent of partial melting²⁰ (high Na_{8.0} for low extents of melting), whereas differences in Fe_{8.0}, CaO/Al₂O₃ and SiO₂ are interpreted as reflecting differences in the depth of partial melting²⁰ (high Fe_{8.0} and CaO/Al₂O₃ and low SiO₂ indicate greater pressure of melting and segregation). Because most of the axial

Table 1 Major-element analyses

Sample no.	9-1	10-8B	12-7	15-4	18-1	21-4	22-5
Location	Seamount flank	Seamount caldera	MAR axis	MAR axis	MAR axis	MAR axis	MAR axis
Oxides							
SiO ₂	49.76	50.75	50.64	51.68	50.90	50.61	51.62
TiO ₂	1.09	1.43	1.62	1.54	1.31	1.09	1.80
Al ₂ O ₃	16.72	15.18	15.42	14.60	15.42	16.16	14.30
FeO							
(total)	8.60	9.63	9.96	10.13	9.08	8.64	11.06
MgO	8.71	7.64	8.05	6.88	7.78	8.62	6.62
CaO	11.98	11.86	11.16	11.95	12.32	12.25	11.13
Na ₂ O	2.44	2.71	2.70	2.85	2.87	2.42	3.02
K ₂ O	0.06	0.07	0.09	0.07	0.08	0.05	0.08
P ₂ O ₅	0.11	0.13	0.14	0.17	0.12	0.10	0.16
Total	99.47	99.40	99.78	99.87	99.88	99.94	99.79
Mg#	68.4	62.9	63.3	59.2	64.7	68.1	56.1

Analyses are by electron microprobe at the Smithsonian Institution. Total iron expressed as FeO, and a ratio Fe₂O₃/FeO = 0.15 was assumed in calculating Mg# (see Fig. 3 legend for definition).

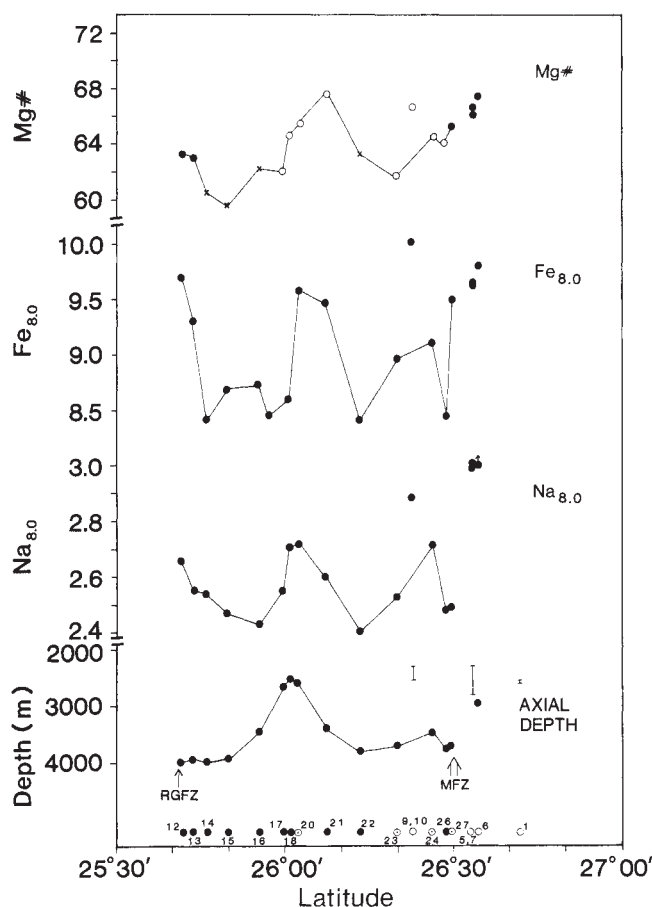


Fig. 3 Axial depth of dredges and chemistry plotted against latitude. The bottom panel shows latitude ($^{\circ}$ S) and depth of dredge hauls; here solid dots represent on-axis hauls, open circles correspond to seamounts on the ridge flanks and circled dots correspond to small cones. Dredges 19 and 25 (slightly off-axis) are not plotted. Plots of $\text{Na}_{8.0}$ and $\text{Fe}_{8.0}$ (see below and ref. 20) are for the most MgO-rich samples of D21 and D22 and averages for the other chemically homogeneous dredges. The plot of $\text{Mg\#}$ ($\text{Mg}/(\text{Mg} + \text{Fe}^{2+}) \times 100$) has symbols coded for the phase assemblages observed in each dredge: $\bullet$, olivine + spinel; $\circ$, olivine + plagioclase; $\times$, olivine + plagioclase + clinopyroxene. The variations of $\text{Mg\#}$, $\text{Fe}_{8.0}$ and $\text{Na}_{8.0}$ exhibit roughly W-shaped patterns, with high values at the offsets and in the centre of the segment. $\text{Na}_{8.0}$ and $\text{Fe}_{8.0}$ are the sodium and iron contents, corrected for the effects of fractionation to an MgO content of 8 wt%: $\text{Na}_{8.0} = \text{Na}_2\text{O} + 0.373(\text{MgO}) - 2.98$; $\text{Fe}_{8.0} = \text{FeO} + 1.664(\text{MgO}) - 13.313$.

lavas at 26° S are unlikely to be primary mantle melts, some fractionation must also be invoked to explain their chemistry. But most melts must have evolved from different, chemically distinct parents, as indicated by Figs 2 and 3. The lack of correlation between $\text{Mg\#}$ and the observed phenocryst assemblages (Fig. 3) and major oxides (Fig. 2) argues strongly against spatially coherent shallow fractionation from a common parental melt. Thus fractionation may be polybaric but must involve numerous, locally available parental liquids.

If these interpretations of $\text{Na}_{8.0}$ and $\text{Fe}_{8.0}$ are correct, as the experimental data suggest, then the depth and extent of melting seem to vary regularly and in a correlated manner along the 26° S segment. It is noteworthy that the off-axis seamounts have systematically high $\text{Mg\#}$, $\text{Na}_{8.0}$ and $\text{Fe}_{8.0}$ (Fig. 3) compared with the axial lavas, suggesting that low extents of melting ($\sim 10\%$)²⁰ at relatively high pressures (>15 kbar)²⁰ occur beneath the ridge-flank seamounts, offsets and near the centre of the segment, with higher extents of melting (up to 13%) at shallower pressures (~ 12 kbar)²⁰ in between.

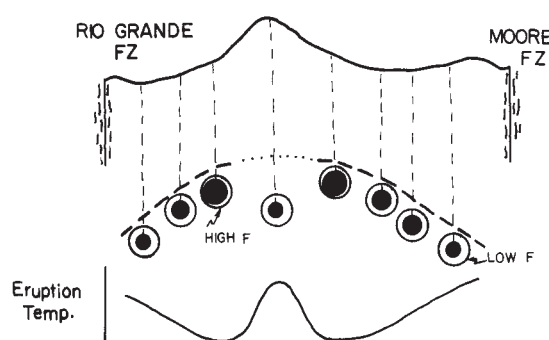


Fig. 4 Cartoon cross-section along the strike of the MAR, showing relative depth of partial melting, relative extent of melting (F) (black shading represents melt) and relative eruption patterns, all inferred from the major-element chemistry of the axial lavas at 26° S. Except for the axial high (anomaly discussed in text), the relative depths of melting define a broad central upwelling, with the extent of melting being low at the edges and higher toward the segment interior. Alternatively, instead of a single broad upwelling, the supply pattern may consist of two separate plumes rising beneath the flanks of the along-axis high. The upwelling regime near the offsets could be an edge effect from diminished vigour of flow near transforms³⁴. Individual melt batches produced at depth then undergo fractionation during transport to the surface. Fractionation (melt temperature) patterns are inferred to be the result of differences in thermomechanical properties at depth, which help to control vertical magma ascent rates.

An alternative possibility is that the observed major-element differences are partly due to melting of a chemically and/or mineralogically heterogeneous mantle. But even if the mantle source supplying the MAR at 26° S is somewhat heterogeneous²¹, it seems very unlikely that this alone could cause such large regular, and especially systematic, variations in the major elements. We therefore propose that correlated differences in the extent and depth of melting of homogeneous mantle are the principal cause of the observed spatially coherent chemical variation patterns. These patterns suggest a magma supply model such as the one shown in Fig. 4. This model features central supply at depth, with multiple injection of individual magma batches.

At $\sim 26^{\circ}$ S, $\text{Na}_{8.0}$ and $\text{Fe}_{8.0}$ are roughly correlated, whereas on a global basis they are roughly anticorrelated^{20,22}. It is not yet known whether other individual MOR segments display such a correlation, but if so, this has important implications for the length scale of the phenomena that control the observed global patterns²². The along-axis depth pattern of the 26° S segment is apparently not uncommon elsewhere in the Atlantic²³⁻²⁷, so if the 26° S segment is chemically representative, the direct correlation of $\text{Na}_{8.0}$ and $\text{Fe}_{8.0}$ may be common in the Atlantic. At faster spreading rates, as along the East Pacific Rise, such patterns have not been observed^{16,28}.

The approximate correlation of $\text{Mg\#}$ with $\text{Na}_{8.0}$ and $\text{Fe}_{8.0}$ (Fig. 3) suggests that the extent of fractionation is correlated with the extent and depth of partial melting. This might be explained by a correlation of the rate of vertical magma ascent and cooling with differences in the thermal regime, as reflected by the depth and extent of melting. This would suggest rapid upward melt transport at the offsets, where cooler conditions may favour more rapid upward propagation of dykes^{29,30}. The presence of high-Mg# lavas at the axial high, however, is not explained by this mechanism. It is also difficult to explain why the axial high, where the most voluminous eruptions have presumably occurred, is not also characterized by the highest extent of partial melting and/or the shallowest depth of melting, as one might expect. One possibility is that the axial lavas are not all the same (that is, zero) age, as has been assumed. Temporal variability or episodic or periodic behaviour of eruptive and

tectonic events^{9,31,32} at any portion of the ridge would be expected to introduce additional complications. We speculate that the surface lavas at the axial high represent the waning stages of a voluminous magma pulse and may be younger, by 10^2 – 10^3 years, than lavas elsewhere along the axis. A similar interpretation has been made recently for the along-axis high at the MAR at 26° N, on the basis of micro-earthquake and refraction data³³.

We gratefully acknowledge the captain, crew, co-chief scientists and scientific party of RC2802 and the ONR for support of this study. We thank P. J. Fox, P. Vogt, E. Klein, C. Langmuir, J. Brodholt, J. Karsten and J. Allan for stimulating discussions.

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Why be female?

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The evolution of dioecy (separate males and females) from hermaphroditism in higher plants has puzzled evolutionary biologists since Darwin¹. An initial step towards the evolution of dioecy may often be the evolution and persistence of females within otherwise hermaphroditic populations^{1–6}. This step is difficult because a nuclear gene for being female must provide a greater than twofold increase in female fitness for females and hermaphrodites to coexist stably^{5–8}. It is unlikely that seed production would increase this much as a result of not producing pollen. Here I show that in buffalo gourd, seeds from females survive their first year in nature 2.8 times more frequently than seeds from hermaphrodites, apparently because seeds from hermaphrodites are mostly self-fertilized and selfing severely reduces seedling survival.

Buffalo gourd, *Cucurbita foetidissima*, a perennial plant native to the southwestern USA and northern Mexico, inhabits disturbed sites, chiefly roadsides. Plants annually produce from one to 30 stems, each up to 12 m long, and can propagate vegetatively by producing roots at leaf nodes. Hermaphrodite (monoecious) plants produce separate male and female flowers, female (gynoecious) plants produce female flowers only. Gender appears to be a mendelian trait, with females the heterogametic sex (refs 9 and 10; and J.R.K., unpublished results). Populations of buffalo gourd contain on average 32% females and 68%

hermaphrodites, and females produce 1.5 times as many seeds as hermaphrodites (J.R.K., in review), an increase which is insufficient to explain their existence, especially at their current frequency^{5–8}. Hermaphrodites are self-compatible, and electrophoretic analysis of seed allozymes indicates that most of their seeds result from self- (or intra-genet) fertilization (J.R.K. and B. B. Casper, manuscript in preparation).

Hand self- and cross-pollinations were performed on hermaphrodite plants in June of 1985. Only one hermaphrodite from any patch was used as a pollen recipient, and cross-pollinations were made using a male flower from another patch to ensure that the pollen was from a different genet. Fruits were collected a minimum of 45 days after pollination and fruits from naturally pollinated flowers of the same hermaphrodite plants and from female plants were also collected. Seeds were removed from the fruits, dried at room temperature and counted.

Among hermaphrodites, cross-pollinations produced more seeds than either self- or natural pollinations, which did not differ (means: cross, 277; self, 184; natural, 168; $n = 15$ plants, $F = 11.6$, $P < 0.001$). Thus a potential cost of self-fertilization is reduced seed set; however, naturally pollinated fruits of females contain 14% fewer seeds than do those of hermaphrodites (J.R.K., in review), even though fruits of females must result from outcrossing. Females may be pollen-limited, but flowers of females are slightly smaller than female flowers of hermaphrodites (J.R.K., in review), so females may also have fewer ovules per flower.

Seed mass did not differ between hand self- and cross-pollinations, but both resulted in lighter seeds than natural pollinations (means, in g per seed: self-, 0.0434; cross-, 0.0415; natural, 0.0473; $F = 5.6$, $P < 0.01$). This may be due to the harvesting of fruits before seeds were completely mature. Under experimental cultivation, buffalo gourd seeds reach full weight and germinability

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Fig. 1 Planting design. Each 0.64-m² array contained either eight naturally pollinated seeds from one of ten females (F) and eight from one of ten hermaphrodites (H), or eight selfed (S) and eight outcrossed (O) seeds from a hermaphrodite, planted at 2-cm depth. Each array containing S and O seeds was planted adjacent to an array containing H seeds from that same individual. Arrays were marked with an aluminium tag at one corner. In each of ten roadside sites, arrays of H and F seeds were replicated five times and arrays of S and O seeds were replicated three times. Because of a shortage of seeds, only three arrays in each site contained naturally pollinated seeds from hermaphrodite number 7, so seeds from another hermaphrodite (number 11) were substituted in the remaining two arrays in each patch. The design called for 1,280 seeds in each of ten sites. Somewhat fewer were planted in sites 6–10 because of seed shortages.

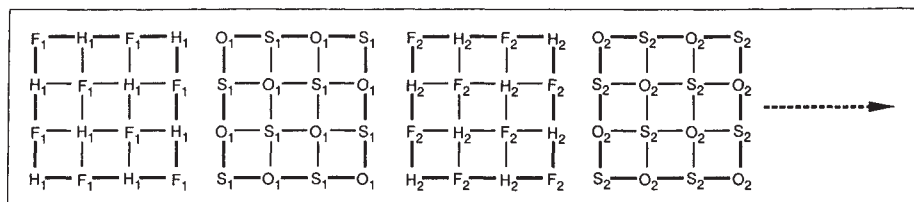


Table 1 Number of surviving plants in August 1987

	Sites	Plants											Total
		1	2	3	4	5	6	7	8	9	10	11	
Self-pollination	2			1		1						—	2
	3											—	0
	4		2	1	1		1		2	1	1	—	9
	5				1	1						—	2
Sub-total		0	2	2	2	2	1	0	2	1	1	—	13
Cross-pollination	2		2	3	1	3				3		—	12
	3	2		1	2	1	1					—	7
	4	2	3	2	4	2	2			2	3	—	20
	5		3		1	1	1					—	6
Sub-total		4	8	6	8	7	4	0	0	5	3	—	45
Hermaphrodite	2				1	2			2	1		1	7
	3					1					2		3
	4	1		4		2			1	1		1	10
	5						1						1
Sub-total		1	0	4	1	5	1	0	3	2	2	2	21
Female	2	1	1	1	2	3			1		2	—	11
	3		2	1	1			1	3			—	8
	4	4	6	9	1	3		3	6		5	—	37
	5				1				1	1		—	3
Sub-total		5	9	11	5	6	0	4	11	1	7	—	59

Only 4 of the original 10 sites produced any survivors.

Table 2 Chi-square values for the association of seedling survival with seed type

Source	d.f.	Self- versus cross-pollination	Hermaphrodite versus female	Self-pollination versus hermaphrodite	Cross-pollination versus female
Intercept	1	486.82†	586.75†	439.26†	738.88†
Seed type	1	15.09†	17.12†	0.11	1.60
Site	3	18.55†	42.26†	15.22*	46.52†

In all comparisons, the association of individual plants with seedling survival and all higher-order associations were not significant and were removed. 0.01 was added to each cell¹⁹.

* $P < 0.01$. † $P < 0.001$.

one month after pollination^{11,12}, but may take longer in nature. Naturally pollinated fruits came from unmarked flowers and may have been older than hand pollinated fruits. Weights of naturally pollinated seeds from females and hermaphrodites do not differ (J.R.K., manuscript in preparation).

The following summer (1986), self-, cross- and naturally pollinated seeds of hermaphrodites and naturally pollinated seeds of females, were planted at 10 sites along the highway where the parental plants live (Fig. 1). Planting sites were far enough from existing patches of *C. foetidissima* that no contamination by unplanted seedlings occurred. Seeds were planted between 1 and 10 July, coincident with the onset of summer rains that cue natural germination. Shortly after planting, rains ceased, and there was severe mortality. It was not possible to measure germination and survival independently because seedlings often blew away after drying up, and some seeds, when dug up, were found to have germinated but desiccated before emergence. Only 26 seeds failed to germinate in 1986, but did germinate in 1987, and these were excluded from the analysis. Seedlings were counted on 15–18 August 1986, and again on 19 August 1987. The length of the longest leaf, a predictor of total leaf area ($r^2 = 0.90$, $P < 0.0001$, $n = 119$ seedlings), was also recorded.

By mid-August 1986, survival of cross-fertilized seeds of hermaphrodites was greatest, whereas self- or naturally pollinated seeds did not differ (means: cross-, 22%; self-, 15%; natural, 15%; $F = 5.2$, $P < 0.05$). Cross-fertilized seedlings were also larger, but the difference was only marginally significant ($F = 3.3$, $P = 0.05$). Survival on this date of naturally pollinated seeds from females was not significantly greater than survival of naturally pollinated seeds of hermaphrodites (means: females, 19%; hermaphrodites, 15%; $F = 1.79$, not significant), nor were there any detectable size differences.

Only $\approx 1\%$ of the seeds produced plants that were still alive in August 1987, but differences in survival had become far more pronounced (Tables 1 and 2). Cross-fertilized seeds survived 3.5 (s.e. 0.8) times more frequently than selfed seeds, and seeds of females survived 2.8 (s.e. 0.6) times more frequently than seeds from hermaphrodites (confidence intervals were jack-knifed¹³). Survival of naturally pollinated seeds of females and cross-pollinated seeds of hermaphrodites did not differ, nor did the survival of self- and naturally pollinated seeds of hermaphrodites. Seeds from females appear to survive better as a result of the benefits of outcrossing, and this difference in survival is large enough to explain the presence of females at their current frequency^{5–8}.

Hermaphrodites self-fertilize most of the time and their seeds suffer severe inbreeding depression. Population genetic models that ascribe the negative effects of selfing to lethal (or strongly deleterious) recessive alleles predict that even moderate selfing should rapidly purge a population of this load^{14,15}. If this were true for buffalo gourd, the advantage females gain through cross-fertilization would soon decline and females would disappear. Recent models can explain the maintenance of substantial inbreeding depression in highly selfed species if inbreeding depression is modelled as resulting from small deleterious effects of genes at many different loci, or similarly as a quantitative trait, or if the benefits of cross-fertilization are due to overdominance^{14,15}. If cross-fertilized seeds survive more than twice as often as self-fertilized seeds, cross-fertilization should be favoured over selfing^{16–18}. If inbreeding depression remains severe despite frequent selfing, outcrossing may remain favoured even when plants are commonly forced to self because of pollinator shortages or inefficiencies.

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Electrogenic glutamate uptake in glial cells is activated by intracellular potassium

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Uptake of glutamate into glial cells in the CNS maintains the extracellular glutamate concentration below neurotoxic levels and helps terminate its action as a neurotransmitter¹. The co-transport of two sodium ions on the glutamate carrier is thought to provide the energy needed to transport glutamate into cells^{2,3}. We have shown recently that glutamate uptake can be detected electrically because the excess of Na⁺ ions transported with each glutamate anion results in a net current flow into the cell⁴. We took advantage of the control of the environment, both inside and outside the cell, provided by whole-cell patch-clamping and now report that glutamate uptake is activated by intracellular potassium and inhibited by extracellular potassium. Our results indicate that one K⁺ ion is transported out of the cell each time a glutamate anion and three Na⁺ ions are transported in. A carrier with this stoichiometry can accumulate glutamate against a much greater concentration gradient than a carrier co-transporting one glutamate anion and two Na⁺ ions. Pathological rises in extracellular potassium concentration will inhibit glutamate uptake by depolarizing glial cells and by preventing the loss of K⁺ from the glutamate carrier. This will facilitate a rise in the extracellular glutamate concentration to neurotoxic levels and contribute to the neuronal death occurring in brain anoxia and ischaemia.

Application of L-glutamate to glial (Müller) cells isolated from the salamander retina evokes an inward current, reflecting electrogenic glutamate uptake⁴. To determine the dependence of this current on internal potassium concentration, we made recordings from voltage-clamped cells, using the whole-cell variant of the patch-clamp technique⁵, with pipettes containing different potassium concentrations (KCl replaced by choline chloride). The potassium concentration inside the cell equilibrates with that in the pipette within about two minutes (assessed from the current changes occurring after patch rupture). The specimen records in Fig. 1a show that when the potassium concentration in the pipette was high the current was large; when the potassium concentration was zero the current was almost abolished. Replacing all the KCl in the pipette by isotonic sucrose instead of choline chloride produced a similar sup-

pression of the glutamate-evoked current, indicating that the suppression is due to removal of potassium rather than the addition of choline. The small glutamate-evoked current which remained when there was no potassium in the pipette may reflect a low level of potassium remaining in the cell resulting from slow leakage from intracellular organelles.

The dependence of the glutamate-evoked current on pipette potassium concentration approximately fits a Michaelis–Menten curve with an apparent K_m of 15 mM (Fig. 1b), implying that the uptake is activated by the binding of one K⁺ ion at the inner surface of the cell membrane. The suppression caused by potassium removal was largely due to an effect on the maximum rate of uptake: removal of internal potassium was not associated with any decrease in the apparent affinity of the carrier for glutamate or sodium at the outer face of the cell membrane (Fig. 2). Indeed, potassium removal produced a small increase in the apparent affinity for these substrates (as predicted, see Fig. 2 legend). This indicates that the activation of glutamate uptake by internal potassium is not a result of allosteric alteration by potassium of the structure of the carrier's binding sites for external glutamate or sodium, to promote binding of these substrates.

If intracellular K⁺ does not just catalyse glutamate uptake, but is actually transported out of the cell by the carrier, we would expect the glutamate-evoked current to be reduced when the extracellular potassium concentration is high. Raising $[K^+]_o$ (substituted for choline) decreased the current, with a 50% reduction occurring at an extrapolated $[K^+]_o$ of 100 mM (Fig. 3). A similar decrease in current was seen when $[K^+]_o$ was raised by adding KCl to the external solution (making it hypertonic) rather than by substitution for choline chloride. The $[Na^+]_o$ -dependence of the current in low and high $[K^+]_o$ indicated that extracellular K⁺ does not reduce the current by occupying the Na⁺-binding sites on the carrier (see Fig. 3 legend). These results are consistent with the idea that potassium is transported out of the cell by the glutamate uptake carrier, and that a raised $[K^+]_o$ prevents the loss of K⁺ from the carrier at the outer surface of the membrane.

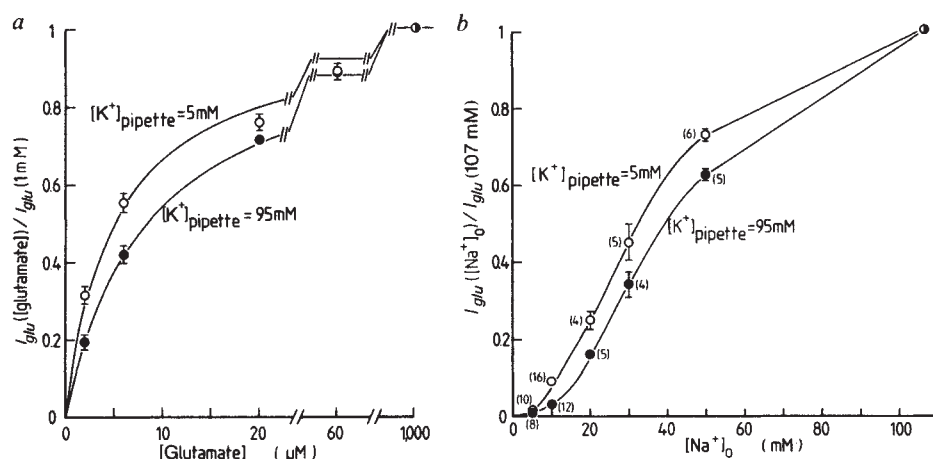
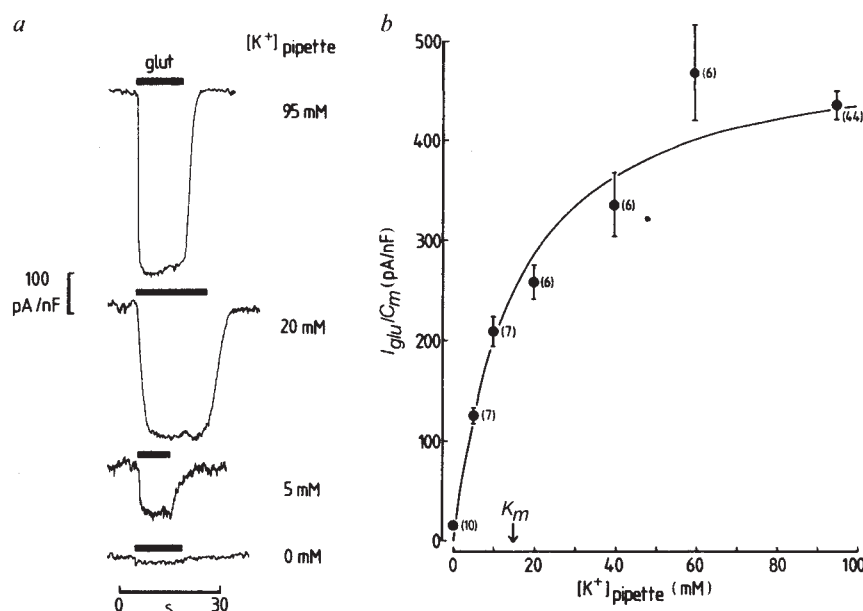
We investigated the possibility of chloride co-transport on the glutamate uptake carrier by replacing all the chloride in the external solution by the much larger gluconate anion (six cells) and, in separate experiments, by replacing all the chloride in the pipette solution by gluconate (six cells studied with each pipette solution). In neither case did chloride removal cause a significant change in the magnitude or voltage-dependence of the glutamate-evoked current. Similarly, removal of external calcium or magnesium (or the barium used to block K⁺ channels) had no effect on the glutamate-evoked current. Changing the external pH away from 7.3 reduced the current, but did not provide conclusive evidence for transport of H⁺ by the carrier (see Fig. 4 legend).

Because glutamate evokes an inward current, there must be a net positive charge transported into the cell during each carrier cycle. Thus, the first-order dependence of the carrier current on internal $[K^+]$ (Fig. 1) and external glutamate concentration (Fig. 2a) and the sigmoid dependence on the external sodium concentration (Fig. 2b) suggest that the simplest stoichiometry possible for this carrier is one where, for each glutamate anion transported into the cell, one K⁺ ion is transported out and three Na⁺ ions (or possibly two Na⁺ and one H⁺) are also transported in (Fig. 4). If only two Na⁺ ions were transported there would be no inward current generated by glutamate (assuming that the form of glutamate transported is the form in which over 99% of it exists at physiological pH, that is, with one net negative charge). The stoichiometry of 3Na⁺:1K⁺:1 glutamate[−] was also suggested by radioactive tracing experiments on rat brain vesicles⁶ where, however, the membrane potential was not controlled (unlike here) making it difficult to be certain that effects attributed to $[K^+]$ changes were not, in fact, produced by membrane potential changes (because the

Methods. Glial (Müller) cells from the tiger salamander retina⁴ were whole-cell clamped using pipettes containing (in mM): KCl, x ; choline chloride $100 - x$; HEPES, 5; MgCl₂, 7; MgATP, 5; CaCl₂, 1; Na₂EGTA, 5; pH adjusted to 7.0 with 14 mM NaOH; [K⁺] = $x = 0, 5, 10, 20, 40, 60$ or 95 mM. Normal external solution (barium-Ringer's) contained (in mM): NaCl, 105; KCl, 2.5; CaCl₂, 3; MgCl₂, 0.5; glucose, 15; HEPES, 5; BaCl₂, 6; pH adjusted to 7.3 with 2 mM NaOH. Barium was included to block most of the potassium conductance of the cell.^{4,10} L-Glutamate was added to the external solution. For these experiments and those in Fig. 2, KCl was omitted from the external solution, because otherwise at negative holding potentials potassium influx prevented the [K⁺] in the cell approaching that in the pipette for low values of [K⁺]_{pipette}, and an apparently weaker dependence of glutamate-evoked current on [K⁺]_{pipette} was observed. Removing external K⁺ has a negligible effect on the glutamate-evoked current when [K⁺]_{pipette} is high (Fig. 3).

glutamate uptake current is extremely voltage-dependent⁴).

The maximum concentration gradient against which a carrier with this stoichiometry can accumulate glutamate is given by $V_{\text{glut}} = 3V_{\text{Na}} - V_{\text{K}} - V_{\text{m}}$ (or $2V_{\text{Na}} + V_{\text{H}} - V_{\text{K}} - V_{\text{m}}$ if 2 Na^+ and H^+ are transported) where V_{glut} , V_{Na} , V_{H} and V_{K} are the Nernst potentials for the ions and V_{m} is the membrane potential. From this we find $[\text{glut}]_i/[\text{glut}]_o \sim 600,000$ (or 42,000 if 2 Na^+ and 1 H^+ are carried) for $V_{\text{Na}} = 50$, $V_{\text{K}} = -95$, $V_{\text{H}} = -17$ and $V_{\text{m}} = -90$ mV. This maximum accumulative power is much greater than that for a carrier co-transporting two Na^+ and one glutamate anion. In this case the maximum $V_{\text{glut}} = 2V_{\text{Na}} - V_{\text{m}}$ and $[\text{glut}]_i/[\text{glut}]_o \sim 1,900$. Most of the extra accumulative power in the former scheme comes from the extra Na^+ (or H^+) transported: in glial cells V_{m} is close to V_{K} and therefore little extra free



energy is provided by transporting K^+ ions across the membrane. Thus, the functional advantage of the counter-transport of K^+ is unclear.

In several pathological conditions in the CNS, such as ischaemia and anoxia (which occur in stroke and perinatal asphyxia), there is massive neuronal depolarization and a large rise in extracellular potassium concentration⁷. Under these conditions the extracellular glutamate concentration can rise to levels that are neurotoxic: too much activation of *N*-methyl-D-aspartate (NMDA)-type glutamate channels leads to an excessive calcium influx (and possibly to osmotic swelling) which kills the neurons^{8,9}. The results presented here and elsewhere⁴ show that the rise in $[K^+]_o$ occurring in these circumstances will reduce electrogenic glutamate uptake, and thus facilitate a rise in glutamate.

Fig. 3 External potassium inhibits electrogenic glutamate uptake. *a*, Current evoked by 30 μ M glutamate (black bars) at -40 mV in a cell that was initially in a solution containing 2.5 mM K^+ , then in a solution containing 57 mM K^+ (which produced an inward current shift of 220 pA through the small fraction of K^+ channels remaining unblocked in 6 mM barium), then again in 2.5 mM K^+ . *b*, Dependence on $[K^+]_o$ of the glutamate-evoked current from experiments like those in *a* (mean data $\pm$ s.e.m. from five cells for $[K^+]_o = 0, 10, 57$ mM and six cells for 25 mM) normalized to the current measured on the same cell with 2.5 mM $[K^+]_o$ (the value of which was set at 0.98 so that the mean value obtained for 0 mM $[K^+]_o$ was unity). External solution contained (in mM): KCl, x ; choline chloride, $57-x$; NaCl, 50; $CaCl_2$, 3; $MgCl_2$, 0.5; glucose, 15; HEPES, 5; $BaCl_2$, 6; pH adjusted to 7.3 with 2 mM NaOH; $[K^+]_o$ was $x = 0, 2.5, 10, 25$ or 57 mM. Pipette solution contained (in mM): KCl, 95; NaCl, 5; HEPES, 5; $MgCl_2$, 5; Na_2 ATP, 5; $CaCl_2$, 1; K_2 EGTA, 5; pH adjusted to 7.0 with 14 mM KOH. Smooth line has the form $K_m/([K^+]_o + K_m)$, with $K_m = 100$ mM. With a near-saturating glutamate dose (1 mM), the current in 57 mM $[K^+]_o$ was 65.2% ($\pm 2.1\%$ s.e.m., five cells) of its size in 2.5 mM $[K^+]_o$, similar to the value in Fig. 3*b* for 30 μ M glutamate ($59.6 \pm 5.8\%$), ruling out the possibility that high $[K^+]_o$ reduces the current by greatly decreasing the carrier's affinity for external glutamate. Reducing $[Na^+]_o$ from 52 to 27 mM (replaced by choline) reduced the current evoked by 30 μ M glutamate at -40 mV by 62.6% ($\pm 1.9\%$ s.e.m., 12 cells) in 2.5 mM $[K^+]_o$ and by 61.2% ($\pm 2.5\%$) in 57 mM $[K^+]_o$, implying an unchanged apparent affinity for external Na^+ in high $[K^+]_o$. If high $[K^+]_o$ reduced the glutamate-evoked current by K^+ competing for the Na^+ binding sites on the carrier, the apparent affinity for Na^+ would be lowered in high $[K^+]_o$ (and reducing $[Na^+]_o$ would give a greater fractional reduction of the current).

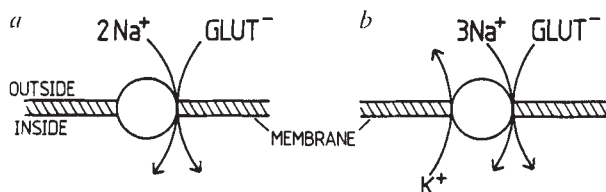
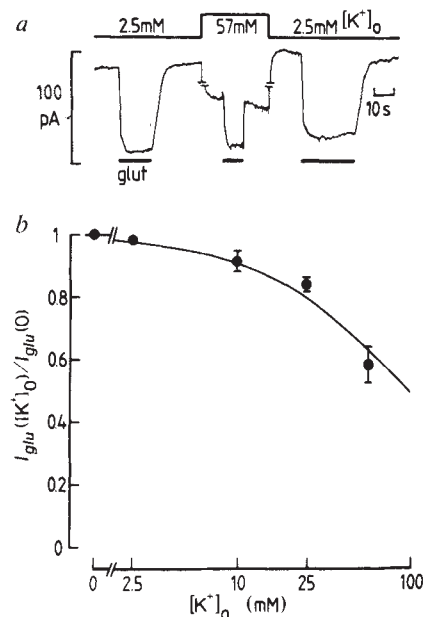


Fig. 4 Stoichiometry of the glutamate uptake carrier. *a*, Stoichiometry based on radioactive tracer studies^{2,3}. Glutamate uptake is driven by the co-transport of two sodium ions into the cell. *b*, Stoichiometry based on the data in this paper. Glutamate uptake is coupled to the transport of one potassium ion out of the cell, as well as to an influx of sodium. We postulate that three sodium ions enter the cell on each carrier cycle: if only two were transported, there would be no current generated. Chloride, calcium and magnesium are not transported (see text). We considered the possibility that protons might be co-transported (so the carrier could transport two Na^+ and one H^+ into the cell instead of three Na^+). At external pH values of 5 or 9 (when 83% of the glutamate exists in the form with one net negative charge, in which $>99\%$ of it exists at pH = 7.3) the glutamate-evoked current was $\sim 25\%$ the size of that at a pH of 7.3. The increase in uptake between pH = 9 and pH = 7.3 could in principle result from the carrier transporting H^+ ions into the cell. But it is equally likely that this pH dependence (and the subsequent decrease on further acidification to pH 5) reflects the titration of charged groups at the binding sites on the carrier for sodium and glutamate.

mate concentration to toxic levels, in two separate ways. First, the raised $[K^+]_o$ will depolarize glia and thus inhibit uptake because of its strong voltage-dependence⁴. Secondly, the rise in $[K^+]_o$ will directly reduce uptake by preventing the loss of K^+ from carrier (Fig. 3). For a rise of $[K^+]_o$ from a physiological 2.5 mM to a pathological 50 mM (as can occur in anoxia⁵) and a consequent glial cell depolarization from around -90 mV to around -20 mV, these mechanisms will reduce uptake by 66% and 35% respectively, a combined reduction to less than one quarter of the physiological rate.

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A transgene containing *lacZ* inserted into the *dystonia* locus is expressed in neural tube

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The site of integration of transgenes in the host genome can affect levels of expression¹ and occasionally confer ectopic patterns of expression on otherwise tissue-specific genes². We describe here a line of mice in which an *hsp68-lacZ* transgene is expressed in unstressed developing neural tissue and where the transgene insertion has caused a mutation of a neural tissue-specific gene, *dystonia musculorum* (*dt*). This coincidence suggests that expression of the *hsp68-lacZ* construct may be controlled directly by *cis*-acting regulatory sequences that normally control the developmental expression of the *dt* gene. Such constructs may serve as useful tools for identifying new tissue-specific enhancers and their associated genes^{3,4}.

We have generated seven lines of transgenic mice carrying a mouse *hsp68* promoter-*Escherichia coli lacZ* hybrid gene for studies of heat-shock gene regulation. In six of these lines the *lacZ* gene was silent unless tissues were subjected to heat shock or arsenite treatment (R.K., S.C., M. Perry, L.A. Moran and J.R., unpublished results). But one line, designated Tg4, expressed the *lacZ* gene spontaneously. This line contained

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Fig. 1 Transgene expression in Tg4/- fetuses. Whole fetuses were stained for β -galactosidase activity. *a*, Day 12.5. Staining is visible as a thin blue line inside the dorsal midline surface of the fetus. *b*, Neural tube and associated DRG from same embryo. An intense band of staining follows the ventral margin of the neural tube. Arrow points to one DRG containing an intense spot of reaction product. *c*, Higher magnification of DRG identified by arrow in *b*. *d*, Day 10.5. Roof of hindbrain vesicle has been removed to reveal twin bands of staining traversing its floor. *e*, Cross-section of day-12.5 neural tube. On either side of the midline the most prominent staining forms a diagonal band between the neural tube's ventral surface and the ventral edge of the developing central canal. A thinner line of staining continues dorsally along the margin of the central canal. *f*, Higher magnification of *e*. In this preparation, most of the reaction product does not appear to lie within cell bodies. At this level of resolution it is not possible to distinguish between staining of cell membranes, fine processes, or extracellular material.

Methods. Whole fetuses were fixed for 1 h in 2.5% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M phosphate buffered saline, pH 7.3 (PBS). They were then incubated from 12–24 h at 37 °C in a solution of 1 mg ml⁻¹ X-gal in PBS (ref. 9). Tissue was viewed as whole mount, or frozen in OCT medium (Tissue-Tek) for cryostat sectioning at 10 μ m.

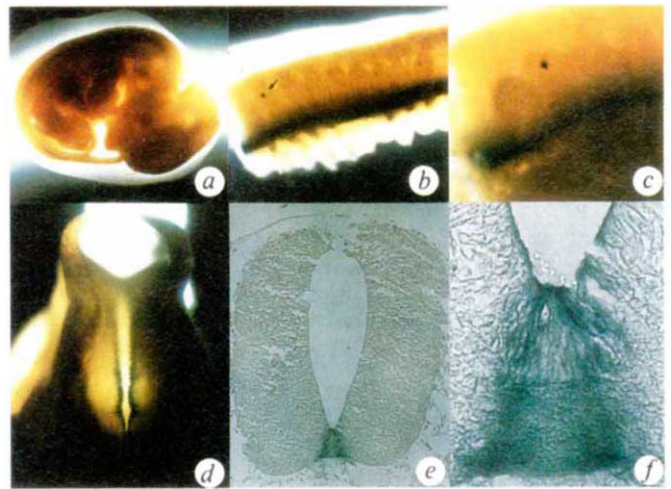
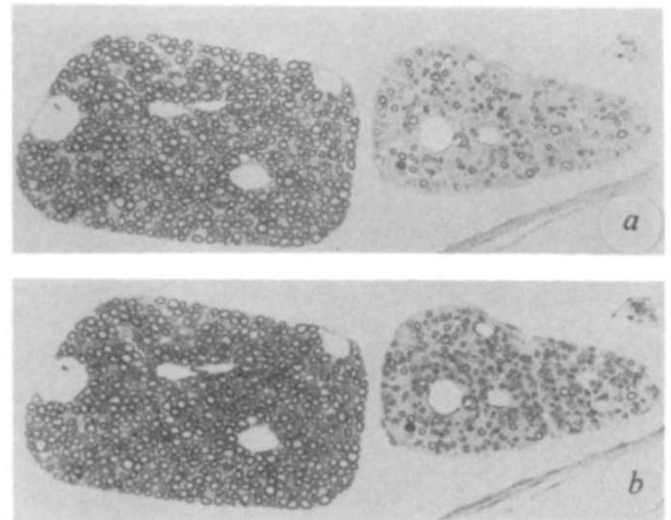


Fig. 2 Cross-sections of spinal roots from the left thoracic 10 (T10) level of *dt^{ts4}/dt⁺* mouse. Normal ventral root is on the left and affected dorsal root is on the right. Serial cross-sections were stained with paraphenylenediamine (*a*) to specifically label myelinated fibres¹⁰ and with toluidine blue (*b*) to reveal general histological features. The small dorsal root contains few myelinated fibres and a large number of apparently denervated Schwann cells. In contrast, the ventral root is of normal size and is composed of myelinated fibers of unremarkable histological appearance.

Methods. Mice were anaesthetized, then perfused through the heart with 0.1 M phosphate buffer (pH 7.2), followed by 2.5% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Spinal roots were embedded in epon, sectioned at 1 μ m and stained. Final magnification $\times 510$.



≈ 15 –20 copies of the transgene inserted as a single head-to-tail concatamer (data not shown). When reacted with X-gal (5-bromo-4-chloro-3-indolyl β -D-galactopyranoside), one-half of the fetuses obtained from normal CD-1 females mated to hemizygous Tg4/- males revealed a column of β -galactosidase activity running along the dorsal midline. Southern blotting of placental DNA confirmed that these embryos were Tg4/- hemizygotes (not shown).

The column of staining was situated along the medial ventral edge of the neural tube and had a triangular shape in cross-section (Fig. 1). Staining was detected first in the rostral-most region of the neural tube in day-9.5 fetuses and was most intense at day 13.5, when staining occurred along the entire length of the neural tube. Also, developing dorsal root ganglia adjacent to labelled levels of the neural tube sometimes expressed one or a few discrete spots of reaction product (Fig. 1). Neonatal mice no longer revealed *lacZ* activity in either spinal cords or ganglia.

When hemizygous Tg4/- mice were mated together, litters of average number were born, but at around 10–12 days after birth one quarter of the offspring began to show signs of limb incoordination. Affected mice progressively lost coordination and most died before weaning or shortly thereafter. Southern blot analysis showed that such affected pups were homozygous for the transgene (data not shown), indicating a recessive inser-

tional mutation. Histological investigation revealed a severe and apparently specific loss of dorsal spinal root sensory axons at all levels of the spinal cord (Fig. 2). Ventral motor roots were remarkably unaffected considering the severe motor symptoms of affected pups. In cross-section, dorsal roots were small, contained few axon profiles, a dramatic paucity of myelinated fibres, and an abundance of apparently denervated Schwann cells. In addition, the occasional giant axon profile was observed.

Both the clinical course of the disease and the histopathological phenotype of this mutant were identical to those described for a spontaneous mutation of the mouse, *dystonia musculorum* (*dt*)^{5,6}. Mice heterozygous for the *dt* allele were obtained from the Jackson Laboratory and crossed with Tg4/- hemizygotes. One quarter of the resulting offspring expressed the typical *dystonia musculorum* phenotype at both the clinical and histopathological levels (Fig. 2), demonstrating that the Tg4 insertional mutant and *dt* are allelic. Generation of a new insertional mutation at the *dt* locus should allow the molecular cloning of the *dt* gene and ultimately lead to the characterization of the *dt* gene product. Further, *dystonia musculorum* has been compared to a group of inherited human neurological diseases, including Friedreich's ataxia, in which an analogous movement disorder is accompanied by sensory neuronal degeneration^{7,8}. Characterization of the mouse *dt* gene may clarify the aetiology of these specific diseases, or provide a more general molecular

model for genetic diseases involving loss of specific neuronal populations.

Insertional mutation of the *dt* gene and the simultaneous appearance of a nervous-system-specific expression profile in the Tg4 transgene line raises the possibility that the two events are directly related. Expression of the *hsp lacZ* construct could be activated by *cis*-regulatory elements of the *dt* gene. The *lacZ* expression profile would, therefore, define the normal developmental and cell type-specific expression pattern of the endogenous *dt* gene. The rare labelled cells we have observed within the developing dorsal root ganglia (DRG) of Tg4 mice may indicate that lack of *dt* gene expression within sensory neurons themselves is directly responsible for the observed neuropathy. Alternatively, the sensory neuropathy could be a secondary consequence of lack of *dt* gene expression within the ventral neural tube where staining was most intense. It is surprising that expression of the *lacZ* gene is obvious only during mid-gestation, whereas degeneration of sensory neurons in *dt/dt* mice apparently occurs post-natally. This may reflect failure of the transgene to respond to all of the elements that normally drive *dt* expression, or it may define a real difference between the time of *dt* gene expression and the time at which deficiency in *dt* products causes an obvious phenotype. There is relatively little information available on the phenotype of *dt/dt* mice before onset of clinical symptoms. The Tg4 insert into the *dt* locus provides a means of identifying homozygotes much earlier in development and it will be interesting to see if subtle phenotypic effects can be detected in the developing neural tube of *dt/dt* mice.

It remains possible that the neural-tube-specific expression of *lacZ* is activated by sequences that are close to the *dt* gene but are otherwise unrelated. In this case, the pattern of *lacZ* expression could identify sequences controlling an unknown gene that could also be important for neural development. Further, as this gene is presumably close to the *dt* locus, the transgene may have inserted into a group of closely linked, neural-specific genes. All these issues will be resolved when the genomic region around the Tg4 insertion is cloned and its products are identified. To this end we are in the process of isolating probes to the flanking sequences of the Tg4 insertion from a genomic library of Tg4/- DNA.

We have clearly shown that a normally inactive promoter, such as *hsp68*, can respond to host regulatory elements in the mammalian genome. It may therefore be possible to design strategies to identify other regulatory elements and their associated genes, exploiting the random insertion of a transgene whose expression can be easily detected *in situ*. Approaches of this sort have begun in both *Drosophila*³ and mice⁴ and could be extremely useful in identifying those genes that participate in the early and fundamental events controlling mammalian embryogenesis.

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The *mas* oncogene encodes an angiotensin receptor

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The class of receptors coupled to GTP-binding proteins share a conserved structural motif which is described as a 'seven-transmembrane segment'¹ following the prediction that these hydrophobic segments form membrane-spanning α -helices. Identified examples include the mammalian opsins², α_1 -, α_2 -, β_1 - and β_2 -adrenergic receptors³, the muscarinic receptor family^{4,5}, the 5-HT_{1C}-receptor⁶, and the substance-K receptor⁷. In addition, two mammalian genes have been identified that code for predicted gene products with sequence similarity to these receptors, but whose ligand specificity is unknown namely, G21 (ref. 8) and the *mas* oncogene⁹. The *mas* oncogene shows the greatest sequence similarity to the substance-K receptor, and on this basis it was predicted that it would encode a peptide receptor with mitogenic activity which would act through the inositol lipid signalling pathways^{10,11}. The *mas* oncogene product was transiently expressed in *Xenopus* oocytes, and stably expressed in a transfected mammalian cell line. The results demonstrate that the *mas* gene product is a functional angiotensin receptor.

A 1.3-kb *Bam*HI/*Nsi*I fragment containing the entire coding region from the human *mas* oncogene⁹ was subcloned into pGEM-7Zf(+). Synthetic RNA was produced and microinjected into *Xenopus* oocytes. Within two to three days, injected oocytes expressed a novel sensitivity not observed in uninjected oocytes. Under voltage-clamp conditions, oocytes injected with *mas*-RNA exhibited a dose-dependent induction of an inward current in response to bath-applied angiotensins I (AI), II(AII), and III(AIII) (Fig. 1a, *n* = 16 oocytes). No response was observed to bath application of bradykinin (100 nM) or to a variety of other peptides. The rank order of potency of mammalian angiotensins was AIII $\geq$ AII $\gg$ AI (Fig. 1b). The responses were reversibly blocked by the broad-spectrum peptide antagonist [D-Arg¹, D-Pro², D-Trp^{7,9}, Leu¹¹]-substance P (ref. 12) (Fig. 1c), but not by the suggested angiotensin antagonists¹³ [Sar¹, Val⁵, Ala⁸]-AII and [Sar¹, Val⁵]-AII, both of which show weak agonist activity (see Fig. 1c). The angiotensin responses clearly displayed the delayed onset and oscillations characteristic of inositol trisphosphate-mediated calcium activation of endogenous chloride channels¹⁴. This implies that the injected *mas*-RNA programmes the expression of angiotensin-sensitive receptors which couple into the endogenous inositol lipid/Ca²⁺-mobilizing pathway. Similar coupling and functional responses have been obtained with expressed serotonin and substance-K receptors^{6,7}.

To extend the functional analysis, a neural cell line NG115-401L, was transfected with the *mas* gene; responses to peptide receptors working through the inositol lipid pathways have been characterized in detail in this system¹⁵, which does not express detectable levels of the *mas* transcript (Fig. 4a). Linearized pMS422 (ref. 9) DNA was co-transfected with pSV2neo by calcium phosphate precipitation. Cells were grown to confluence in the presence of G418 to select for transfectants, and pooled populations screened for angiotensin sensitivity using the intracellular calcium indicator, Fura-2 (ref. 16). These cells were then cloned by limiting dilution, and a number of individual

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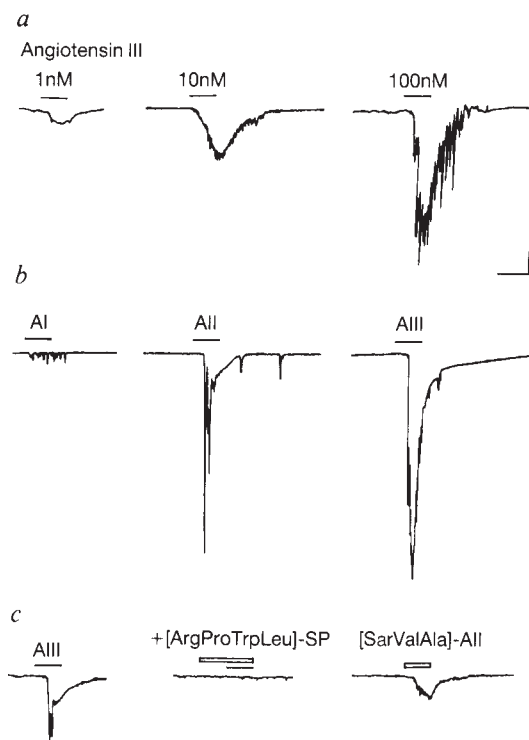


Fig. 1 Angiotensin responses in *Xenopus* oocytes injected with synthetic *mas* RNA. *a*, Nanomolar concentrations of angiotensin III evoked dose-dependent currents. Bars above traces indicate duration of drug applications. Calibration bars: vertical, 100 nA (*a*), 50 nA (*b*, *c*); horizontal, 1 min. *b*, Relative potency of angiotensins I, II and III. The responses to 100 nM AI (left) were significantly smaller than those induced by 100 nM AII or AIII (centre and right). Recordings are from a single oocyte, different from the one shown in *a*. *c*, Effects of peptide-receptor antagonists tested in an oocyte injected 3 d earlier with *mas* RNA. Left, current response induced by 10 nM AIII. Centre, application of 10 μ M (D-Arg¹, D-Pro², D-Trp^{7,9}, Leu¹¹)- substance P (open bar) reversibly inhibited the response to 10 nM AIII (solid bar). Right, the putative angiotensin II receptor antagonist [Sar¹, Val⁵, Ala⁸]-AII (open bar, 10 μ M) weakly activated the receptor.

Methods. A 1.3-kilobase (kb) *Bam*HI/*Nsi*I fragment containing the entire coding region of the *mas* gene was subcloned into pGEM-7ZF(+). The plasmid was linearized by digestion with *Nsi*I and transcribed *in vitro*²⁴ using T7 RNA polymerase. Transcripts were capped with m⁷G(5')ppp(5')G (ref. 25). The synthetic RNA gave a single band of ~1.3 kb on a 1.5% formaldehyde gel. RNA was injected into defolliculated *Xenopus* oocytes at a concentration of 1 ng nl⁻¹ (volume injected per oocyte, ~50 nl) and incubated at 17 °C for 1.5–3 days in Barth's medium supplemented with 2.5 mM pyruvate. Physiological recordings were made at 20–23 °C using standard two-electrode voltage clamp techniques. Oocytes were continuously perfused with frog Ringer saline (~2 ml min⁻¹; volume of recording chamber, 0.5 ml). Drugs were bath-applied for 45 s and oocytes were rinsed for 5–20 min between drug applications. Uninjected oocytes showed no response to 100 nM angiotensin I, II or III.

angiotensin-responsive clones were obtained. One of these, 401L-C3, was selected for detailed characterization. By Northern blot analysis, it expressed a 2.4-kb transcript when probed with a 0.35-kb *mas* probe (Fig. 4*a* and *b*). Figure 2*a* shows the responses of transfected and parental populations. The dose-response profiles for cytosolic [Ca²⁺]_i elevation in 401L-C3 cells (Fig. 2*b*) exhibited the same rank order as found for oocyte current stimulation: AIII ≥ AII > AI, suggesting that this pharmacology is an intrinsic feature of the receptor. In Fig. 3*a*, the pattern of cytosolic calcium changes is compared with those induced by bradykinin. It can be seen that although the maximal

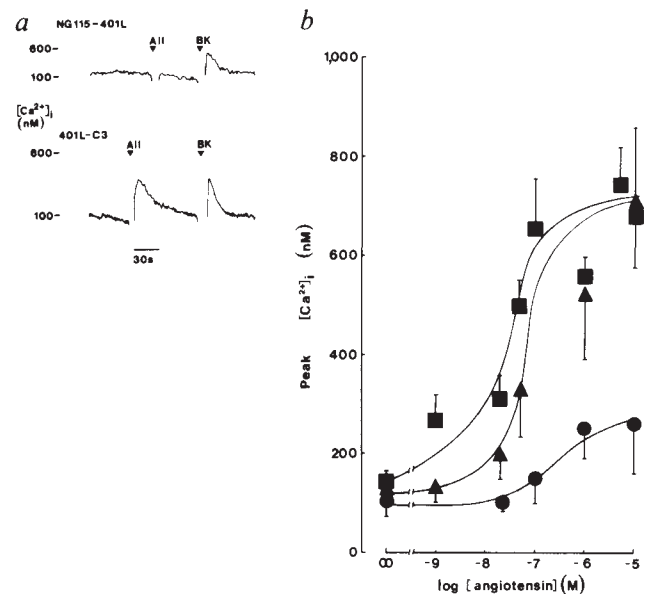


Fig. 2 *a*, Intracellular Ca²⁺ transients in parental NG115-401L or transfected 401L-C3 cell populations in response to angiotensin II (AII, 1 μ M) and bradykinin (BK, 2 μ M). *b*, Dose-dependence of peak intracellular calcium concentration [Ca²⁺]_i responses for angiotensin I (●), II (▲) or III (■) in 401L-C3 cell populations. **Methods.** *Sma*I linearized pMS422 and *Pst*I linearized pSV2neo were co-transfected into NG115-401L cells by calcium phosphate precipitation²⁶. Cells were grown to confluence in the presence of G418 (1.8 mg ml⁻¹, Gibco), and pooled G418^r cell populations were screened for angiotensin sensitivity by [Ca²⁺]_i responses. Individual angiotensin-responsive clones were then obtained by limiting dilution. For [Ca²⁺]_i determinations, cells were grown on coverslips and loaded with Fura-2/AM; [Ca²⁺]_i was monitored by Fura-2 fluorescence at 500 nm with excitation at 340 nm, and calibrated using digitonin and manganese chloride²⁷. In *a*, traces are representative of at least three separate determinations; in *b*, each data point represents mean $\pm$ s.e.m. for 3–9 separate determinations.

response to angiotensin II is similar to bradykinin, it shows slower kinetics (~5 s and 15 s to peak respectively). Pre-treatment of the cells with bradykinin can abolish the response to angiotensins, but pre-treatment of the cells with angiotensins attenuates, though does not abolish, the response to bradykinin. Like the oocyte response, angiotensin stimulation of an intracellular calcium transient can be blocked by the previously described peptide receptor antagonist, unlike the bradykinin-induced transient. These two responses can also be distinguished by their sensitivity to acute phorbol diester treatment. Figure 3*b* indicates that acute treatment with TPA (12-*O*-tetradecanoylphorbol-13-acetate) can attenuate the response evoked by AII, but is without effect on that evoked by bradykinin. This result indicates that protein kinase C may act directly on either the *mas* gene product or a transduction component, such as a G-protein, which is used by this receptor and not by the bradykinin receptor. These conclusions about the intracellular calcium transients elicited by angiotensins have been reproduced and extended using single-cell imaging approaches (T.R.J. *et al.*, manuscript in preparation).

As the *mas* oncogene has been shown to be tumorigenic in nude mice, and has transforming activity on NIH 3T3 cells⁹, we examined the ability of angiotensins to stimulate DNA synthesis in the 401L-C3 clone. As Table 1 shows, angiotensins stimulate [³H]thymidine incorporation into serum-starved cells, whereas bradykinin does not.

We have examined the tissue distribution of the *mas* proto-oncogene transcript in normal human brain and in peripheral tissues. As shown in Fig. 4*c*, the highest level of the transcript

Table 1 [^3H] thymidine incorporation in parental NG115-401L and transfected 401L-C3 cell populations

NG115-401L addition	[^3H]Thymidine % control	401L-C3 addition	[^3H]Thymidine % control
Control	100	Control	100
10 μM BK	103 $\pm$ 3	10 μM BK	103 $\pm$ 10
10 μM AI	ND	10 μM AI	126 $\pm$ 7*
10 μM AII	105 $\pm$ 10	10 μM AII	155 $\pm$ 23*†
10 μM AIII	ND	10 μM AIII	158 $\pm$ 20*
% FCS	257 $\pm$ 8*	5% FCS	294 $\pm$ 13*†

[^3H]Thymidine incorporation into serum-starved parental NG115-401L and transfected 401L-C3 cells. Cells were plated at low density ($\leq 1 \times 10^4$ cells per cm^2) in 35-mm tissue culture dishes in DMEM containing 5% FCS; 1 h later the medium was replaced with serum-free DMEM, then after 24 h cells were stimulated as appropriate in the presence of 0.5 $\mu\text{Ci ml}^{-1}$ [^3H]thymidine (New England Nuclear). After 48 h further incubation, the radioactivity incorporated into trichloroacetic acid-insoluble material was assessed by liquid scintillation counting¹². Data are means $\pm$ s.e.m. of at least three separate determinations each performed in triplicate. Radioactivity present in controls: NG115-401L 15,469 $\pm$ 1,567 c.p.m.; 401L-C3 15,209 $\pm$ 735 c.p.m. Significance was determined by a two-tailed Student's *t*-test.

* Different from control, $P < 0.05$.

† 401L-C3 different from NG115-401L, $P < 0.05$. ND, not determined.

was found in human cortex, with lower amounts in hippocampus and cerebellum, similar to the results found for rat brain¹⁷. The *mas* transcript was expressed at very low levels in human liver, kidney and adrenal glands. These results are consistent with the anticipated peripheral distribution of an angiotensin receptor. There may exist a multigene family whose protein products respond to angiotensins, and there may be one or more receptors for each of the naturally occurring molecular forms of angiotensins (AI, AII and AIII). Earlier observations¹⁸ reported that both brain angiotensin-binding sites and functional responses appeared to prefer AIII over AII. Thus, the *mas* oncogene may encode a 'neuronal-type' angiotensin receptor.

The identification of the *mas* proto-oncogene product as an angiotensin receptor with mitogenic activity strongly suggests that at least some neural peptides may function as growth factors^{19,20} and thus could be important during the development of the nervous system. It also indicates that deregulation of the inositol lipid signalling pathways could, in some circumstances, be sufficient for tumorigenesis. It is interesting to note that the location of the human *mas* gene on chromosome 6 (ref. 21) is within a region of fragile sites associated with neuroblastoma and other tumours. Little is known of the activation of the *mas* proto-oncogene, but earlier work⁹ suggested that the proto-oncogene itself had intrinsic tumorigenic capacity. Rearrangement of the 5'-flanking sequence confers enhanced tumorigenicity. Additionally, the human *mas* oncogene shows only weak transforming activity in NIH 3T3 cells, but is paradoxically quite potent in eliciting tumours *in vivo*. This result may now be explicable with the realization that tumour induction may require stimulation of the *mas* gene product by its cognate ligand, an angiotensin. Accordingly, the actions of exogenously administered angiotensins or suitable antagonists in promoting or retarding *mas*-induced tumour formation *in vivo* should now be assessed. This is the first oncogene for which a defined pharmacology, including potential antagonists, is available.

These results are also interesting in a pathological context different from carcinogenesis. For some time, angiotensin dysfunction has been proposed as a possible mechanism in genetic and experimental hypertension²². Recent hypotheses on the origin of essential hypertension²³ have emphasized the likely role of vascular hypertrophy following chronic exposure to an endogenous growth factor. It is possible that the endogenous growth factor could be an angiotensin.

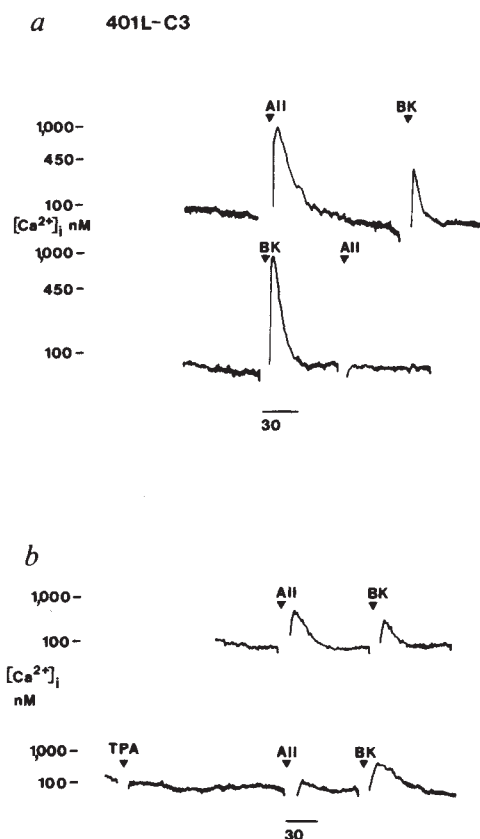


Fig. 3 *a*, Intracellular Ca^{2+} transients in response to angiotensin II (AII, 10 μM) and bradykinin (BK, 2 μM) in 401L-C3 cell populations. *b*, Effect of acute TPA (200 nM) treatment on $[\text{Ca}^{2+}]_i$ responses to angiotensin II (AII, 1 μM) and bradykinin (BK, 200 nM) in 401L-C3 cell populations.

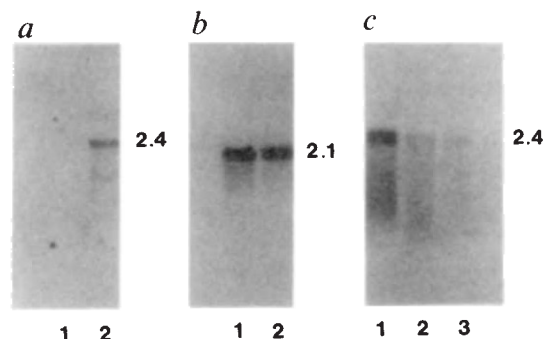


Fig. 4 *a, b*, Northern blot analysis of poly(A)⁺ RNA from parental NG115-401L (lane 1) and transfected 401L-C3 (lane 2) using ^{32}P -labelled human *mas* oncogene DNA (*a*) or mouse β -actin DNA (*b*) as a probe. The blot was hybridized with the actin probe following dehybridization of the *mas* probe. *c*, Northern blot analysis of poly(A)⁺ RNA from control human brain using ^{32}P -labelled human *mas* oncogene as a probe. Lanes: 1, frontal cortex; 2, hippocampus; 3, cerebellum.

Methods. A 0.35-kb *Kpn*I/*Sal*I fragment from the *mas* oncogene clone pMS422 (ref. 8) was subcloned into M13mp18 and single-stranded DNA probes prepared²⁸. Human tissues were obtained less than 3 h after death. RNA extractions, hybridizations and washings were performed as described previously²⁸. Each lane contained 20 μg poly(A)⁺ RNA. The autoradiographic exposure times were 6 d (*a*), 1 h (*b*) and 5 d (*c*). The 0.24–9.5-kb RNA ladder (Bethesda Research Laboratories) served as a size marker.

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Note added in proof: The G-21 clone has now been shown to encode a 5-HT_{1A} receptor²⁹.

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***Bcl-2* gene promotes haemopoietic cell survival and cooperates with *c-myc* to immortalize pre-B cells**

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A common feature of follicular lymphoma, the most prevalent haematological malignancy in humans, is a chromosome translocation (t(14;18)) that has coupled the immunoglobulin heavy chain locus to a chromosome 18 gene denoted *bcl-2* (refs 1-6). By analogy with the translocated *c-myc* oncogene in other B-lymphoid tumours (reviewed in ref. 7), *bcl-2* is a candidate oncogene, but no biological effects of *bcl-2* have yet been reported. To test whether *bcl-2* influences the growth of haematopoietic cells, either alone or together with a deregulated *c-myc* gene, we have introduced a human *bcl-2* complementary DNA (ref. 3) using a retroviral vector into bone marrow cells from either normal or *Eμ-myc* transgenic mice, in which B-lineage cells constitutively express the *c-myc* gene⁸⁻¹⁰. *Bcl-2* cooperated with *c-myc* to promote proliferation of B-cell precursors, some of which became tumorigenic. To determine how *bcl-2* expression impinges on growth factor requirements, the gene was introduced into a lymphoid and a myeloid cell line that require interleukin 3 (IL-3) (refs 11 and 12). In the absence of IL-3, *bcl-2* promoted the survival of the infected cells but they persisted in a G₀ state, rather than proliferating. These results argue that *bcl-2* provided a distinct survival signal to the cell and may contribute to neoplasia by allowing a clone to persist until other oncogenes, such as *c-myc*, become activated.

The retroviral constructs (Fig. 1a) use the MPZen vector¹³; one contains only a *bcl-2* cDNA and the other also bears the selectable marker *neo*^R (ref. 13). Virus-producing lines were isolated by transfecting the ψ 2 fibroblast packaging line¹⁴. Neither the ψ 2 cells nor infected NIH 3T3 cells were altered morphologically.

To determine whether *bcl-2* affects normal haemopoietic cells and to test for synergy with *c-myc*, bone marrow cells from preneoplastic *Eμ-myc* mice or normal littermates were co-cultivated with ψ 2 cells producing the MPZen(*bcl-2*) virus and then cultured in soft agar (see legend to Fig. 1). No lymphoid colonies (<1 per 10⁶ cells) arose from either infected normal marrow or mock-infected *Eμ-myc* marrow, but the infected *Eμ-myc* marrow yielded compact colonies (67 per 10⁶ cells plated) containing non-adherent cells with a lymphoid morphology. They survived for several weeks in liquid culture but did not proliferate, even in the presence of a stromal support cell line for lymphoid progenitors¹⁵ or of haemopoietic growth factors IL-1, IL-2, IL-3, IL-4, IL-5, IL-6 or GM-CSF.

Bcl-2 also cooperated with *c-myc* to promote proliferation of pre-B cells in liquid culture. In three experiments in which infected marrow cells were seeded into liquid medium, normal cells survived for only a few weeks, as did mock-infected normal or *Eμ-myc* cells, but the infected *Eμ-myc* marrow cells continued to proliferate, albeit slowly (doubling in five days) (Fig. 1b). All three infected *Eμ-myc* populations, which express the expected viral *bcl-2* message, now appear to represent immortal lines as they have proliferated autonomously for at least 130 days. All have a pre-B lymphocyte phenotype as they display rearranged immunoglobulin heavy chain loci but no surface immunoglobulin. Significantly, two of the three lines tested after eight weeks of culture yielded tumours in nude and syngeneic mice within seven weeks after injection of 10⁶ cells. Although the growth curves (Fig. 1b) suggested that many infected pre-B cells must have proliferated initially, continued selection was evident from the simple patterns of immunoglobulin gene rearrangements and viral inserts found after 10 weeks of culture (data not shown). The oligoclonality of these lines and the failure of the agar clones to proliferate in liquid medium suggest that even enforced expression of *bcl-2* plus *c-myc* is not usually sufficient for autonomous growth and tumorigenicity.

To determine whether *bcl-2* altered the growth factor requirements of established haemopoietic cell lines, the *bcl-2/neo*^R virus was used to infect the pro-B cell line LyH7 (ref. 11) and the myeloid line FDC-P1 (ref. 12), both of which die if deprived of IL-3. Infected or mock-infected clones were picked from agar, expanded in IL-3 containing medium, washed three times and then cultured for four days without factor. All LyH7 and FDC-P1 clones harbouring the *bcl-2* virus contained many live cells; the average viability of nine clones of each tested was 62 ± 3% and 59 ± 5% respectively, whereas that of mock-infected clones was only 6.5 ± 1% and 0 ± 0%. Although none of 40 *bcl-2*-bearing clones tested proliferated without factor, all grew normally after its readdition, even several days after withdrawal. Thus *bcl-2* expression does not render FDC-P1 cells factor independent. Nor does it make them tumorigenic, because syngeneic mice injected with 10⁶ cells from 20 *bcl-2* virus-bearing lines did not develop tumours within six months.

The growth-arrested status of factor-derived LyH7 and FDC-P1 clones bearing the *bcl-2* virus was confirmed by flow cytometric analysis of their viability, cell size, nuclear size and DNA content, as illustrated for FDC-P1 in Fig. 2. Whereas less than 2% of uninfected FDC-P1 cells survived three days without factor, clones harbouring the MPZen(*bcl-2*) virus remained 90% viable (compare first panel of Fig. 2b and d; dead cells are shaded). Significantly, the infected cells were much smaller in the absence of IL-3 (compare first panel of Fig. 2c and d). Their DNA content revealed that most had ceased to cycle; less than 4% were in the S or G₂/M phases, compared with 30% in the same clone cultured with factor (compare Fig. 2d with c). The

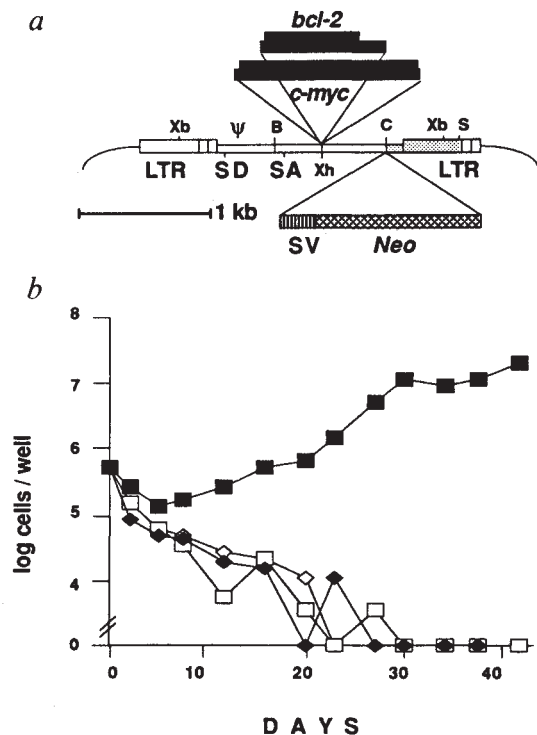


Fig. 1 *a*, pMPZen retroviral constructs. Open areas derive from pZipNeoSV(X) (ref. 25); filled areas represent cDNAs for *bcl-2* and *c-myc*. The region from the myeloproliferative sarcoma virus (MPSV) (ref. 26) is stippled, stripes indicate the SV40 early promoter (SV), and hatching the neomycin-resistance gene (Neo)¹³. The packaging signal is denoted by ψ (ref. 14), and SD and SA are the splice donor and acceptor respectively. Restriction endonuclease sites are Xh, XhoI; Xb, XbaI; C, ClaI; B, BamHI; S, SacI. *b*, Effect of *bcl-2* on growth of bone marrow cells. ■, Eμ-myc marrow initially cultured on MPZen(*bcl-2*) virus-producing fibroblasts; ◆, Eμ-myc marrow on ψ2 fibroblasts; □, normal marrow on MPZen(*bcl-2*) ψ2 fibroblasts; ◇, normal marrow on ψ2 fibroblasts. Viable cells (those excluding eosin) were counted microscopically.

Methods. The vector pMPZen was constructed by removing the XhoI fragment from pZipNeoSV(X) (ref. 25) and replacing the ClaI-SacI fragment of the 3'LTR with that from MPSV (ref. 26); to produce pMPZenSVNeo, an SVNeo cassette²⁷ was inserted into the ClaI site¹³. A blunted EcoRI/TaqI fragment of the human *bcl-2* cDNA (ref. 3) was introduced into the XhoI site of pMPZen and pMPZenSVNeo. The insert encodes the entire 239-amino-acid polypeptide, but not the putative 205-amino-acid polypeptide predicted by some workers⁵. Transfection¹⁶ of ψ2 fibroblasts¹⁴ was with retroviral DNA and, where required, pSV2Neo. G418-resistant ψ2 clones were expanded and screened for a provirus of the correct size by Southern blot analysis of XbaI digests. MPZenSVNeo(*bcl-2*) ψ2 clones were titred by scoring the ability of filtered supernatants to confer G418 resistance on NIH 3T3 fibroblasts¹⁶; the clone used has a titre of 1.0×10^5 per ml. DNA analysis of cells co-cultivated with the MPZen(*bcl-2*) ψ2 line showed that 5 of 12 FDC-P1 clones harboured a provirus of the correct size, indicating an infection rate of 40%. Bone marrow cells from 4-week-old mice of the Eμ-myc line 292-1 (ref. 10) or their normal littermates were suspended in Dulbecco's modified Eagle's medium and $1-2 \times 10^6$ viable cells added to a monolayer of 2×10^6 irradiated (35 Gy) MPZen(*bcl-2*) ψ2 cells in 5-ml medium containing 10% fetal calf serum (FCS), 10^{-4} M 2-mercaptoethanol (2-ME) and $20 \mu\text{g ml}^{-1}$ lipopolysaccharide. After 48-72 h, the non-adherent cells were collected, washed and plated in 0.3% agar or liquid culture in the absence of mitogenic stimulus. Agar colonies were scored microscopically after 7 d before cytocentrifugation and staining or transfer to liquid medium containing 10% FCS, 10^{-4} M 2-ME and growth factors (see text).

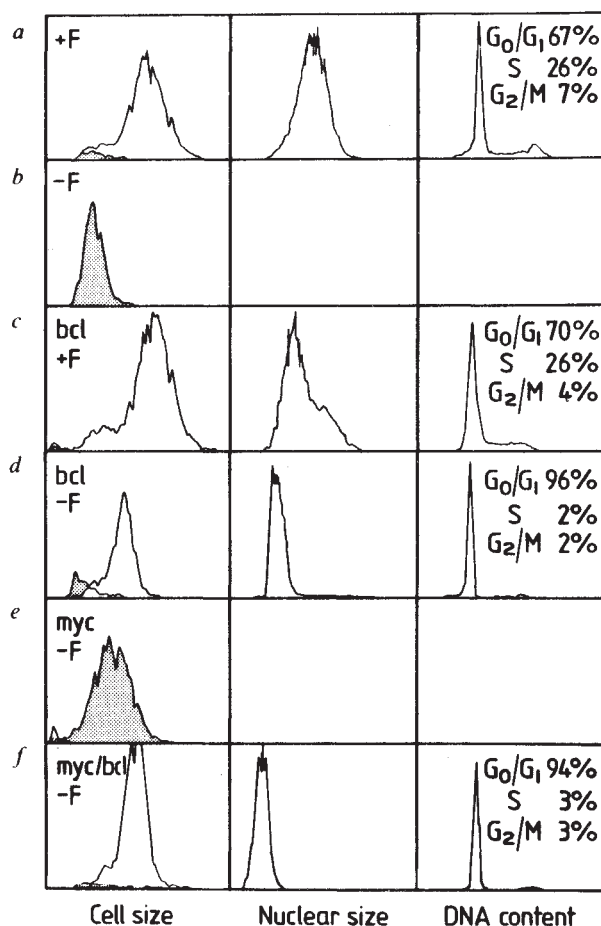


Fig. 2 Flow cytometric analysis of FDC-P1 clones cultured with or without factor. The left column shows cell-size profiles, those of dead (propidium iodide (PI)-stained) cells being shaded. The right column shows histograms of DNA fluorescence. The nuclear size profiles (middle column) were generated from nuclei that had a G₀/G₁ DNA content by DNA fluorescence. *a*, FDC-P1 cells with factor; *b*, FDC-P1 cells without factor; *c*, *bcl-2* FDC-P1 cells with factor; *d*, *bcl-2* FDC-P1 cells without factor; *e*, *c-myc* FDC-P1 cells without factor; *f*, *c-myc/bcl-2* FDC-P1 cells without factor.

Methods. Washed cells cultured for 3 d in the presence or absence of factor were analysed on a modified FACS II (Becton-Dickinson). Forward low-angle light scatter was used to resolve cells or nuclei on the basis of size, and dead cells were detected by PI uptake. To determine the percentage of cells in replicative cycle, cells were lysed with 2% Triton X-100 and their nuclei stained with $4 \mu\text{g ml}^{-1}$ PI (ref. 28). The fraction of cells in the S plus G₂/M phases of the cell cycle was derived from the single parameter profile of red fluorescence²⁹.

nuclei comprising the G₀/G₁ peak were much smaller in *bcl-2*-expressing cells cultured without factor than in any of the proliferating cultures (compare nuclei in Fig. 2*d* with *a*). This suggests that the deprived cells were in G₀ rather than G₁.

As *c-myc* expression is strongly associated with cell proliferation⁷⁻⁹, it is not surprising that FDC-P1 cells bearing the *bcl-2* virus expressed the 2.3-kilobase (kb) *c-myc* messenger RNA while proliferating (Fig. 3*b*, lane 2), but not when deprived of IL-3 (lane 3). To test whether enforced *c-myc* expression could drive their proliferation, we infected 12 such clones with the *c-myc* retrovirus MPZenSVNeo(*c-myc*) (see Fig. 1*a*). The proliferating drug-resistant clones expressed the ~5-kb viral *c-myc* transcripts (Fig. 3, lane 4) at a level high enough to suppress the endogenous *c-myc* gene (see ref. 7), but when starved of factor they survived in a G₀ state (Fig. 2*f*) and did not proliferate. LyH7 cells bearing both viruses behaved similarly. Hence the concerted action of *c-myc* and *bcl-2* does not render either cell

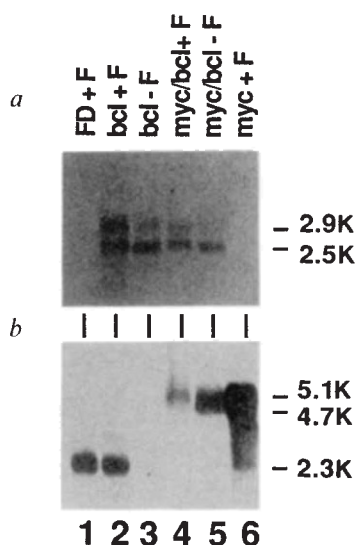


Fig. 3 Northern blot analysis of poly(A)⁺ mRNA from FDC-P1 clones with a *bcl-2* probe (a) and a *c-myc* probe (b). Lane 1, FDC-P1 cultured with factor; lane 2, *bcl-2* FDC-P1 with factor; lane 3, *bcl-2* FDC-P1 without factor; lane 4, *c-myc/bcl-2* FDC-P1 with factor; lane 5, *c-myc/bcl-2* FDC-P1 without factor; lane 6, *c-myc* FDC-P1 with factor. The 2.9-kb genomic and 2.5-kb spliced *bcl-2* transcripts expected from the MPZen(*bcl-2*) provirus (see Fig. 1a), the 5.1-kb genomic and 4.7-kb spliced transcripts from the MPZenSVNeo(*c-myc*) provirus and the 2.3-kb endogenous *c-myc* transcript are indicated.

Methods. Poly(A)⁺ mRNAs (5 µg) prepared¹⁶ from cells grown for 3 d with or without factor were heated at 65 °C for 5 min in buffered 2 M formaldehyde/50% formamide, fractionated electrophoretically on a 1% agarose/2 M formaldehyde gel and blotted onto nitrocellulose. Filters were hybridized with randomly primed probes (5 × 10⁶ c.p.m. ml⁻¹) in 5 × SSC (SSC is 0.15 M NaCl, 15 mM sodium citrate, pH 7.0), 50% formamide at 42 °C and washed in 0.2 × SSC at 65 °C.

type autonomous. The biological action of *c-myc* differs from *bcl-2*, because FDC-P1 cells containing only the *c-myc* virus all died when deprived of factor (Fig. 2e), consistent with evidence that *c-myc* can reduce but not abolish growth factor requirements¹⁶.

The results presented here indicate that *bcl-2* can contribute to neoplasia, but suggest that its action is relatively subtle. A *bcl-2* retrovirus did not morphologically transform NIH 3T3 fibroblasts, immortalize cells from normal bone marrow, or render FDC-P1 cells tumorigenic. Unlike *v-abl*, polyoma middle T and *v-fms* oncogenes^{17,19}, *bcl-2* did not allow FDC-P1 (or LyH7) cells to proliferate without factor. It did, however, promote the eventual immortalization of B lineage cells constitutively expressing *c-myc* (Fig. 1b), and it prevented the death of FDC-P1 and LyH7 cells starved of factor.

The unique ability of *bcl-2* to dissociate cell survival from proliferation suggests that a haematopoietic growth factor provides two signals: one keeps the cell alive, the other stimulates division. Our results suggest that the *bcl-2* protein acts within the 'survival' signal pathway, whereas *c-myc* lies on that involved in proliferation. Although short-term survival of IL-3 dependent

cells is promoted by phorbol ester, calcium ionophore, glucose and ATP (refs 20 and 21), *bcl-2* allows prolonged survival (for at least one week) with nearly all the cells remaining in G₀.

The biological properties of *bcl-2* described here are consistent with the indolent early stages of follicular lymphoma, where the cells have a normal morphology and non-invasive growth patterns²³. As many B-lineage cells have a short half-life²⁴, a major effect of the constitutive *bcl-2* expression induced by the 14;18 translocation may simply be to maintain the affected clone until other genetic alterations, which sometimes involve *c-myc* translocation²⁴, generate the more aggressive later stages. Our finding that simultaneous expression of *bcl-2* and *c-myc* within lymphoid progenitors predisposed to their immortalization and eventual tumorigenesis provides direct evidence for *c-myc/bcl-2* synergy within the relevant cells.

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Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors

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The T-cell repertoire is elaborated by a still poorly understood process during which precursor cells arising in the bone marrow seed the thymus to provide a starting point for intrathymic differentiation and selection. The products of the process are cells which express antigen receptors composed of either α/β or γ/δ heterodimers in association with CD3 (refs 1–3). The finding that the appearance of T-cell antigen receptor γ - and δ -gene rearrangements and transcripts precedes those of full-length β - and α -transcripts during ontogeny indicates that the process is ordered^{4–8}, a conclusion supported by the fact that the appearance of thymocytes expressing CD3-associated γ/δ heterodimers precedes the appearance of those bearing α/β heterodimers⁹. The recent demonstrations that within the γ - and δ -loci there is ordered and sometimes transient rearrangement and expression of specific V δ and V γ gene segments during ontogeny^{7,8,10} raised the possibility that qualitative changes in the capacity of the differentiative process to generate components of the T-cell armamentarium might occur. We have produced a monoclonal antibody that detects an epitope of the V γ 3 gene product¹⁰, a gene segment expressed only in the early fetal thymus¹¹. In this report we demonstrate that cells expressing V γ 3 are present transiently at the earliest stages of thymocyte development, preceding the appearance of cells bearing other γ/δ or α/β receptors. In the adult mouse, V γ 3 expression appears to be limited to Thy-1⁺ cells in the epidermis. These results suggest a profound programming and staging in elaboration of the components of the T-cell system during the early stages of thymocyte development in the embryo.

The epidermis of normal adult mice contains Thy-1⁺, CD4⁸ dendritic cells which express a CD3-associated γ/δ heterodimer^{12–14}. We have found that the genes encoding the receptor chains of each of five independent Thy-1⁺ dendritic epidermal cell (dEC) clones derived from AKR mice are composed of V γ 3/J γ 1/C γ 1 (refs 14 and 15) and V δ 1/D δ 2/J δ 2/C δ segments (D. Asarnow *et al.*, unpublished results). It is interesting that both V γ 3 and V δ 1 transcripts appear to be expressed preferentially, if not exclusively, during the earliest stages of thymocyte development^{7,11}. To study T-cell antigen receptor (TCR) expression by emerging thymocytes, we have produced a monoclonal antibody (mAb) to the γ/δ TCR using a Thy-1⁺ dEC clone as immunogen. The antibody, mAb 536, binds to and stimulates interleukin-2 secretion by the Thy-1⁺ dEC clones, but not lines that express α/β TCR, and immunoprecipitates the γ/δ heterodimer. Analysis of a panel of hybridomas characterized for V γ and V δ gene usage indicated that the antibody is directed to an epitope on the V γ 3 gene product¹⁰.

As seen from Fig. 1a, three-colour flow cytometric analysis shows that mAb 536 reacts with essentially all Thy-1⁺ dEC freshly isolated from the trunk epidermis of BALB/c mice. These results confirm that the TCR γ -chain repertoire of Thy-1⁺ dEC is restricted to the V γ 3 gene segment. Similar analysis of T cells in the adult thymus, spleen and lymph nodes failed to show the presence of cells expressing V γ 3 (Fig. 1b–d). There was no evidence of the presence of CD3⁺ cells reactive with mAb 536 in an analysis of CD4⁸ thymocytes and T splenocytes, which should be enriched for γ/δ TCR-bearing cells, from normal adult BALB/c mice¹⁰. Essentially identical results were obtained with Thy-1⁺ dEC and lymphoid tissues from AKR and C57BL/6 mice. Thus it appears that expression of V γ 3 in the adult animal is largely, if not entirely, restricted to cells in the epidermis.

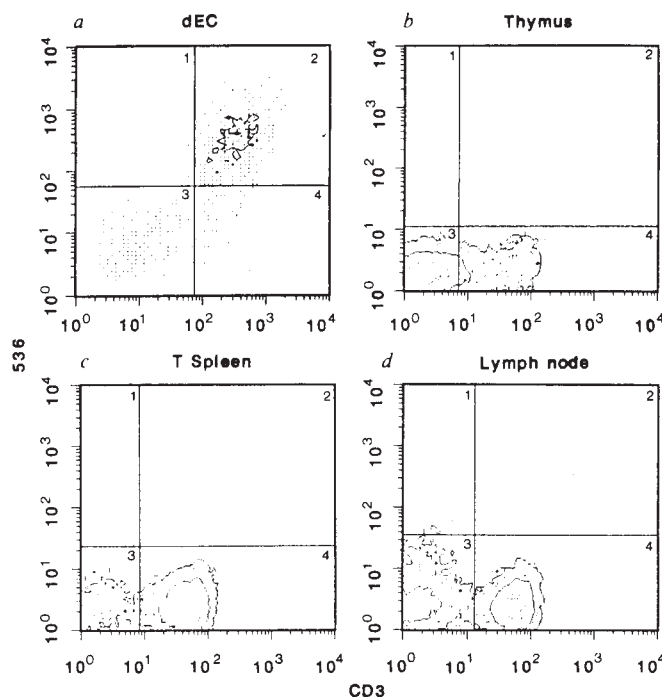


Fig. 1 Multi-colour FACS analysis of the expression of CD3 and V γ 3 on adult BALB/c lymphoid tissues. *a*, Epidermal sheets were removed, dissociated by digestion with trypsin, and cells isolated by Ficoll-hypaque density gradient centrifugation¹⁹. Interface cells were incubated with biotin-anti-Thy-1 (mAb HK2.1) (ref. 20), FITC-anti-CD3 ϵ (mAb 500A2) (ref. 21), and arsenyl (ARS)-derivitized anti- γ/δ TCR (mAb 536) (ref. 10) for 1 h at 4 °C. After washing, allophycocyanin-avidin and phycoerythrin anti-ARS were added for an additional hour at 4 °C. Electronic gates were set to resolve Thy-1⁺ cells, which made up 35% of the total population, and data displayed as two-colour contour plots of CD3 and mAb 536 binding. Cells were analysed on a FACS 440 system with an argon ion laser (488 nm) and a helium neon laser (633 nm) (Becton Dickinson Immunocytometry Systems). Data analysis was performed using the Consort 30 system (Becton Dickinson). *b–d*, Thymus, spleen and mesenteric lymph node were removed and live lymphoid cells isolated by Ficoll-hypaque banding. Splenocytes were enriched for T cells by panning on anti-immunoglobulin coated flasks. The non-adherent population was 97% Thy-1⁺. 3×10^5 cells were incubated with FITC-anti-CD3 and ARS-anti- γ/δ TCR, as described in *a*.

Table 1 Expression of TCR on fetal thymocytes

Time	Number of mice	Total cells per thymus	% CD3 ⁺	% of CD3 ⁺ 536 ⁺	F23.1 ⁺
Fetal thymus					
day 14	18	2.5×10^4	6.8	88.9	<1
day 15	15	8.4×10^4	4.9	74.1	<1
day 16	30	2.6×10^5	11.1	53.3	<1
day 17	10	4.9×10^5	13.8	30.2	3.4
day 18	9	6.7×10^5	19.9	<1	8.1
day 19	5	9.6×10^5	23.3	<1	15.9
day 20	9	2.8×10^6	31.4	<1	17.8
Newborn					
day 1	8	1.6×10^6	48.4	<1	24.7
day 2	12	7.1×10^6	53.9	<1	25.6
4-week adult	8	6.1×10^7	61.7	<1	24.5

Three-colour FACS analysis was performed on BALB/c thymocytes using FITC-500A2 (anti-CD3 ϵ), biotin-F23.1 (anti-V β 8 TCR), and arsenyl-536 (anti-V γ 3 TCR). Cells were incubated for 1 h at 4 °C with these mAb, then washed twice. Phycoerythrin-anti-ARS and allophycocyanin-avidin were then added for an additional hour and cells analysed as described in the legend to Fig. 1. Results are representative of a minimum of three separate experiments for each time point on BALB/c or C57BL/6 thymocytes.

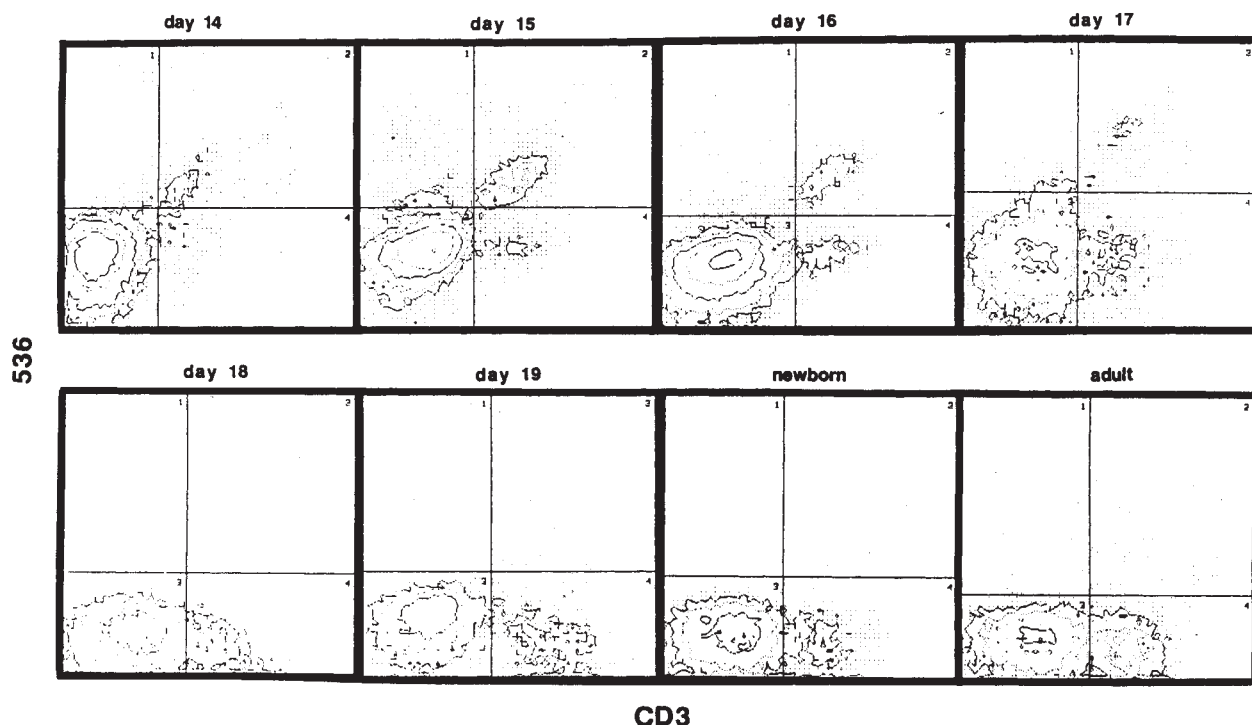
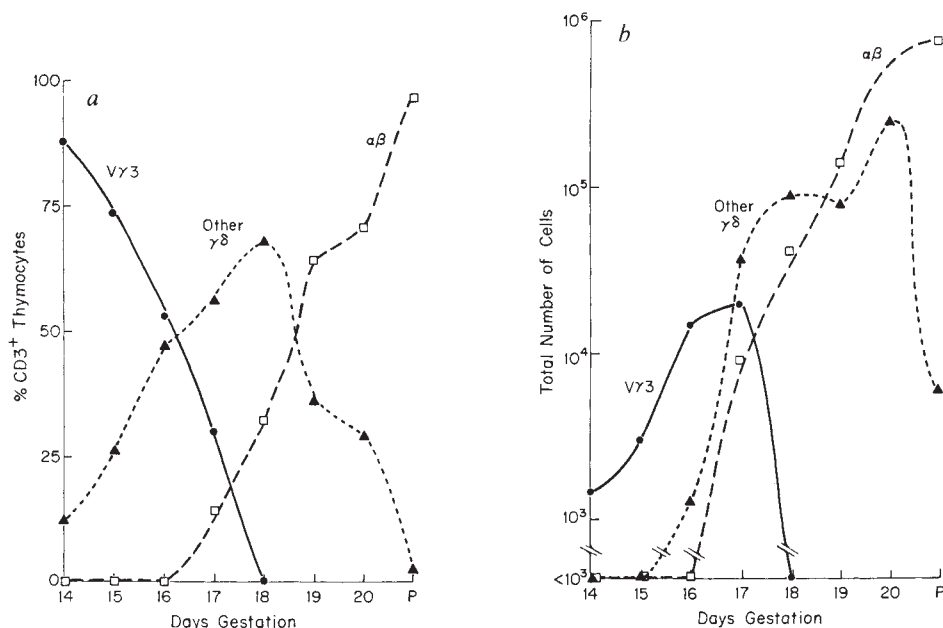


Fig. 2 Two-colour FACS analysis of the expression of CD3 and $V\gamma 3$ on BALB/c fetal thymocytes. Thymuses were removed daily from embryos obtained from timed pregnant mice on day 14 of gestation through birth. Single-cell suspensions were obtained and enriched for live lymphoid cells by Ficoll-hypaque density centrifugation. Immunofluorescence analysis was performed as described in Fig. 1.

Fig. 3 Expression of TCR chains during fetal development. Data presented in Table 1 were plotted as percentage of $CD3^+$ thymocytes (*a*) and as total number of cells found in the thymus (*b*) on days 14 of gestation through to parturition (*p*). The values for $V\gamma 3$ expression represent actual data points. Multiplication of the number of mAb F23.1+ ($V\beta 8$) cells by four gave an approximation of the total number of α/β TCR-bearing cells present at each time point. Subtraction of the sum of this value and the number of 536+ cells from the total number of $CD3^+$ cells provided an estimate of the number of cells using other γ/δ TCR chains. The dotted line for other γ/δ cells and the dashed line for α/β cells represent extrapolated values and not actual data points.



Another group has reported that a subset of dEC lines obtained from C3H/HeJ ear epidermis express $V\gamma$ gene products distinct from $V\gamma 3$ (refs 12 and 13). Analysis of ear epidermis showed $V\gamma 3$ expression by >85% of $Thy-1^+$ dEC from AKR, C57BL/6 and BALB/c mice, whereas <50% of $Thy-1^+$ dEC from C3H/H3J mice expressed $V\gamma 3$ (data not shown). C3H/HeJ trunk epidermis also showed decreased $V\gamma 3$ expression (~75% of cells reacted with mAb 536) and we are investigating this apparent strain difference.

To assess expression of $V\gamma 3$ by cells in the developing thymus, fetal, newborn and adult thymocytes were examined for reactivity with mAb 536 and anti-CD3 by flow cytometric analysis.

As shown in Fig. 2, most $CD3^+$ thymocytes express $V\gamma 3$ at days 14 and 15 of fetal development. The abundance of $V\gamma 3^+$ cells decreases as fetal development continues, and $V\gamma 3^+$ cells are undetectable after day 17. Numerical data for the analysis are presented in Table 1. Eighty-eight per cent of the $CD3^+$ thymocytes from day-14 fetal mice expressed $V\gamma 3$, suggesting that these cells might be the first TCR-bearing cells to arise in ontogeny. But the presence of $V\gamma 3^+$ cells in the thymus appears to be transient, because no significant $V\gamma 3$ fraction was detectable after day 17.

To more fully assess TCR expression during ontogeny, thymocytes were analysed by three-colour flow cytometry using

anti-CD3, mAb 536, and mAb F23.1. The F23.1 antibody detects an epitope of the V β 8 gene product and reacts with ~25% of mature α/β T cells¹⁶. As shown in Table 1, F23.1⁺ cells were not detectable until day-17 of gestation and increased to a maximum fraction of CD3⁺ cells by birth. Assuming that V β 8 is expressed by 25% of α/β T cells at all stages of development, we used a multiplication factor of four to approximate the number of cells expressing α/β TCR in our analysis. The difference between total CD3⁺ cells and the sum of V γ 3⁺ and estimated α/β cells was used to estimate the fraction of cells that express γ/δ TCR using V γ segments other than V γ 3. The data from Table 1 and the extrapolated values are presented graphically in Fig. 3 as a percentage of total CD3⁺ cells (Fig. 3a), or as approximate numbers of cells (Fig. 3b) expressing V γ 3/ δ , other γ/δ TCR, or α/β TCR as a function of gestational time.

It is apparent from Fig. 3 that there are at least four waves of appearance of cells bearing TCR in the developing thymus. The first to appear are cells that express V γ 3, which decrease steadily as a fraction of CD3⁺ cells, being replaced by thymocytes bearing γ/δ TCR not encoded by the V γ 3 gene, and which comprise most of the CD3⁺ cells on days 17 and 18 (Fig. 3a). Thymocytes bearing α/β TCR appear at day 17 and comprise most of the CD3⁺ cells in the thymus after day 19 (Fig. 3a). Since the size of the thymus increases exponentially during fetal development, we also examined the contributions of each of the three classes of cells to the total number of cells (Fig. 3b). It is apparent that a third peak of cells expressing non-V γ 3 γ/δ TCR occurs at day 20 of gestation.

The data presented here demonstrate that the ordered expression of TCR genes^{4,5,11} is reflected by the sequential appearance of distinct cell populations bearing the products of those genes during thymocyte development. It has been reported that γ - and δ -gene rearrangements during fetal ontogeny are not cumulative, suggesting the occurrence of a major change in the populations of cells present in the thymus⁷. Our data suggest that this change is a result of the death or emigration of the first wave of cells expressing TCR composed of the V γ 3 gene product. Although a lineal relationship has not been firmly established, it is reasonable to propose on the basis of our findings that this first wave of CD3⁺ cells emigrate to seed the skin, giving rise to the Thy-1⁺ dEC in adults. The fetal origin of Thy-1⁺ dEC in the adult is supported by our observation (D. Asarnow *et al.*, unpublished results) that non-germ line encoded nucleotides are essentially absent from the junctions of the rearranged γ - and δ -genes of Thy-1⁺ dEC clones; this is consistent with the absence of non-germ line encoded nucleotides from the junctions of rearranged δ -genes isolated from the fetal, but not from the adult, thymus^{7,8}. It is interesting that the γ/δ TCR of Thy-1⁺ dendritic cells occurring in intestinal epithelium¹⁷ appear to use restricted V γ and V δ genes which are distinct from V γ 3 (C. Janeway, personal communication) and might be the descendants of the second wave of cells seen in the thymus. A mAb directed against the avian homologue of the mammalian γ/δ TCR has recently been described¹⁸. In studies using this mAb, similar waves of TCR expression were seen in chicken embryonic thymus¹⁸.

Taken together, our findings indicate that generation of components of the immune system is temporally programmed by the order of TCR gene rearrangement, and suggests that the periphery is seeded with discrete populations of T cells generated at different stages in development. It also appears that the capacity of the immune system to generate at least one class of cells, the dEC, is absent in the fully mature thymus. Whether this is due to changes in the thymic microenvironment or to alterations in the machinery of rearrangement remains to be determined.

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Lymphocyte activation by HIV-1 envelope glycoprotein

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Cell activation by phytohaemagglutinin, phorbol ester and by the supernatant of phytohaemagglutinin-stimulated peripheral blood mononuclear cells induces the expression and cytopathic effects of latent human immunodeficiency virus type-1 (HIV-1) *in vitro*¹⁻³. The lymphocyte surface protein CD4 has been identified as a receptor for HIV-1^{4,5} and binds the viral envelope glycoprotein (gp120)^{6,7}. In the light of evidence indicating that one natural function of CD4 is as a growth factor receptor⁸⁻¹⁰, we examined the ability of native gp120 to activate resting CD4-bearing lymphocytes. Our results indicate that gp120 has innate biological activity as a result of a specific interaction with CD4, inducing increases in intracellular levels of inositol trisphosphate and of calcium, and in interleukin-2 receptor expression and cell motility.

The CD4 protein, originally defined by monoclonal antibodies against antigens for T-lymphocyte membrane differentiation^{11,12}, has the capacity to alter multiple T-cell functions related to antigen recognition and signal transduction. There is good evidence that CD4 enhances T-cell activation by serving as a receptor for class II major histocompatibility (MHC) molecules present on stimulator or target cells¹³⁻¹⁶. Related studies¹⁷⁻²⁰ indicate that the presence of CD4 on the cell surface enhances the affinity of the T-cell antigen receptor for antigen, possibly by binding to class II MHC proteins on presenting cells. Structural considerations²¹, and the internalization and phosphorylation of CD4 after phorbol ester or antigen stimulation^{8,22,23}, suggest that the CD4 protein may also function as a growth factor receptor, independent of class II MHC molecules⁸⁻¹⁰. In this context, we examined the ability of the CD4 ligand gp120 to stimulate intracytoplasmic signalling mechanisms and to activate resting CD4-bearing lymphocytes.

Lymphocyte activation by antigen and anti-CD3 antibody acts through a common signal pathway with the generation of

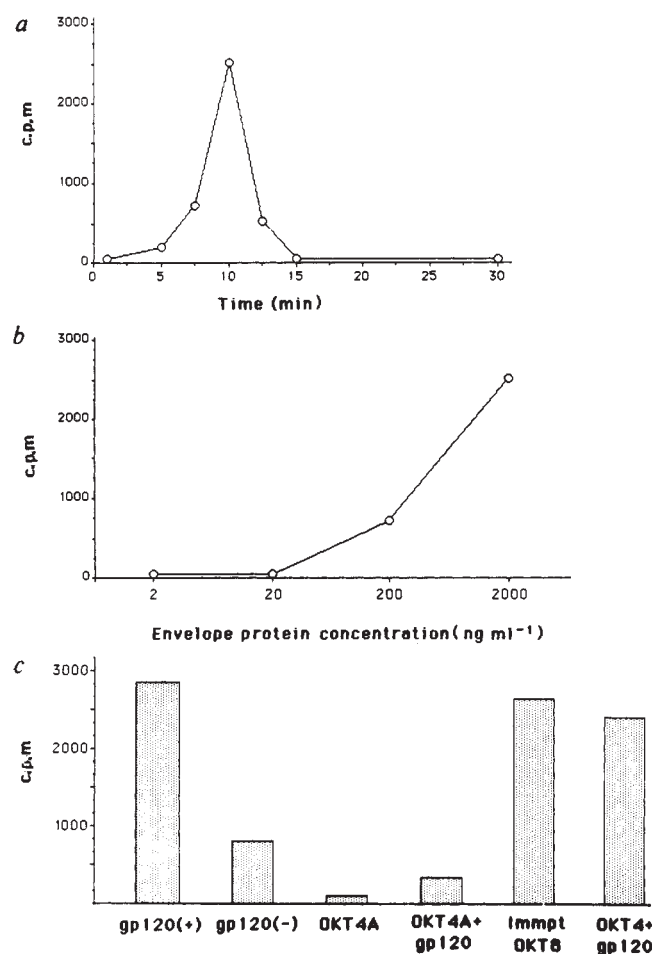


Fig. 1 The envelope glycoprotein of HIV-1 increases intracellular IP₃ levels. *a*, Time course of the gp120 effect on IP₃ levels. Myo[2-³H] inositol-labelled T cells were incubated with 2.0 µg ml⁻¹ envelope protein, corresponding to ~10⁻⁸ M gp120. The reaction was stopped at the time points indicated and IP₃ measured as described below. *b*, Dose-response of the gp120 effect on IP₃. IP₃ levels were measured in cells incubated with different dilutions of gp120-containing supernatant for 10 min. *c*, Specificity of the gp120 effect on IP₃. IP₃ levels were measured after 10 min in cells incubated with 2.0 µg ml⁻¹ gp120 (gp120(+)), gp120 immunoprecipitated with anti-gp120 (gp120(-)), or gp120 immunoprecipitated with OKT8 antibody (Immpt OKT8). In addition, cells were stimulated with OKT4A alone (OKT4A), or stimulated with gp120 following pre-incubation with OKT4A (OKT4A + gp120) or OKT4 (OKT4 + gp120) antibody.

Methods. Nylon wool-nonadherent T cells were prepared from peripheral blood of HIV-1 seronegative volunteers⁴⁷ and incubated overnight in medium 199 with 0.4% bovine serum albumin. Cells were then incubated for three hours at 37 °C with 40 µCi myo[2-³H]inositol per 20 × 10⁶ cells. Aliquots of 20 × 10⁶ cells were then incubated as above. At the indicated time points, inositol phosphates were isolated from cell lysates and identified by HPLC^{48,49}. Results are expressed as (c.p.m. minus background) per 10⁷ cells. Immunoprecipitation of gp120-containing viral supernatant was performed by binding goat anti-gp120 raised to acrylamide gel-purified gp120³¹ or OKT8 antibody, to protein A-Sepharose CL-4B beads (Pharmacia) for 1 h at room temperature, washing, then incubating with gp120 to 1 h at room temperature.

inositol triphosphate (IP₃) from membrane phosphoinositides, which acts to release calcium from intracellular stores²⁴⁻²⁹. We therefore measured intracellular IP₃ and calcium levels in nylon wool non-adherent T cells prepared from peripheral blood of healthy HIV-1-seronegative donors after stimulation with gp120. The gp120 was prepared from the supernatant of HIV-1-infected

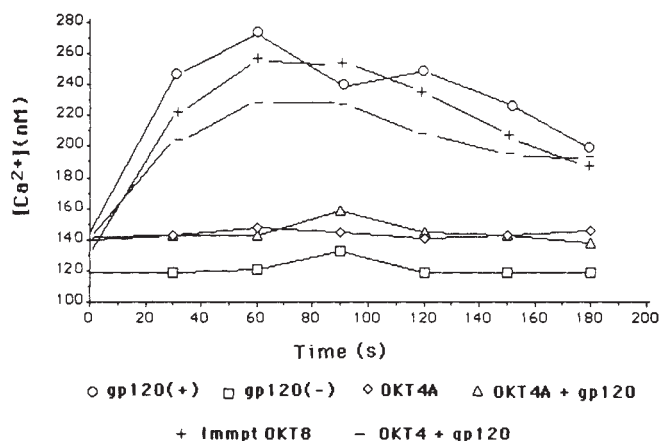


Fig. 2 The envelope glycoprotein of HIV-1 increases intracellular calcium levels. Indo-1-loaded T cells were exposed to 2.0 µg ml⁻¹ gp120, gp120 immunoprecipitated with anti-gp120 sera or OKT8 antibody, OKT4A antibody alone, or pre-incubated with OKT4A or OKT4 antibody and exposed to gp120. Intracellular calcium was measured by fluorimetry.

Methods. Intracellular calcium levels were measured with the calcium probe indo-1. T cells were incubated in phosphate-buffered saline (pH 7.4) with 5 mM glucose and 5 µM indo-1 for 30 min 37 °C. Intracellular fluorescence was monitored by a Perkin-Elmer 650-10S spectrofluorimeter with an excitation wavelength of 355 nm, and emission wavelengths of 485 nm and 405 nm for calcium-free and calcium-bound signals respectively. Calcium concentrations were calculated as described³⁴.

H9 cell cultures by immunoaffinity chromatography³⁰. The purified supernatant was ~50% gp120, as estimated by polyacrylamide gel electrophoresis and Western blotting. The results are expressed as the protein concentration of the purified supernatant (2.0 µg ml⁻¹ corresponds to ~1 × 10⁻⁸ M gp120).

For IP₃ measurements, cells were metabolically labelled with myo[2-³H] inositol and [³H]IP₃ was quantitated in cell lysate fractions eluted from an HPLC anion exchange column. As shown in Fig. 1*a*, gp120 caused a transient rise of IP₃, peaking at 10 min after stimulation. Increased IP₃ production occurred with 0.2 µg ml⁻¹ gp120 and rose more than 10 times above basal levels at 2.0 µg ml⁻¹ gp120 (Fig. 1*b*). Thus, this activity of gp120 is observed at concentrations close to the reported dissociation constant of recombinant gp120 for CD4 ($K_d = 4 \times 10^{-9}$ M; ref. 7). To demonstrate that the observed effect is due to gp120, immunoprecipitation was performed using goat anti-gp120³¹ bound to protein A-coated Sepharose beads. The immunoprecipitated material had significantly reduced activity (Fig. 1*c*), whereas incubation with protein A alone, or protein A plus a neutral (OKT8) antibody did not reduce the activity of gp120. A preparation of partially purified p24 from HIV-1-infected H9 cells was also without activity in this system. Pre-incubation of cells with OKT4A antibody significantly inhibited the IP₃ response to gp120, but OKT4 had no blocking activity (Fig. 1*c*). This is consistent with competition between OKT4A, but not OKT4, and gp120 for CD4 binding^{32,33}. None of the anti-CD4 antibodies alone stimulated IP₃ production.

Intracellular calcium ([Ca²⁺]_i) measurements were conducted with the calcium probe indo-1 (ref. 34). As shown in Fig. 2, an increase in [Ca²⁺]_i from 140 to 270 nM occurred within 60 s of stimulation with 2.0 µg ml⁻¹ gp120. The rise in calcium was reduced by ~15% if cells were stimulated in the presence of 1 mM EDTA, indicating that most of the calcium increase was due to release from intracellular stores. Neither anti-gp120-immunoprecipitated supernatant nor OKT4A induced a calcium response. Cells pre-incubated with OKT4A, but not with OKT4, failed to respond to gp120.

To determine whether the observed intracytoplasmic signals generated by gp120 were associated with activation of resting (G_0) T cells, we analysed interleukin-2 receptor (IL-2R) expression with fluorescent monoclonal anti-IL-2R antibody. Resting T cells express minimal levels of IL-2R and increased expression of this receptor occurs with progression from the G_0 to G_{1a} phase of the cell cycle³⁵. We found that $2.0 \mu\text{g ml}^{-1}$ gp120 induced increased surface expression of IL-2R on resting T cells so that the percentage of positive cells rose from less than 5% to 18% at 24 h (Fig. 3a), reaching levels of up to 26% at 48 h (Fig. 3b). As with calcium and IP_3 measurements, the effects of gp120 were blocked by immunoprecipitation of the viral supernatant or by pre-incubation of cells with OKT4A antibody (Fig. 3b).

Experiments in our laboratory have demonstrated that T-lymphocyte growth factors interleukin-2 (IL-2), insulin, and insulin-like growth factor-1 (IGF-1) are all capable of inducing a motile response in lymphocytes³⁶⁻³⁸, suggesting that this activity may be a general response of lymphocytes to growth factors. We therefore tested gp120 in chemotaxis experiments using a modified Boyden-chamber technique³⁹. An increase in motility of more than 200% of the control was observed at gp120 concentrations similar to the IP_3 and IL-2R responses (Fig. 4a). A motile response was seen with T cells and enriched populations of CD4-bearing cells, but not with CD8-bearing cells, or if the gp120 was immunoprecipitated with anti-gp120 (Fig. 4b). Limited checkerboard analysis⁴⁰ indicated that the motile response to gp120 was chemotactic (directed), rather than chemokinetic (random) in nature (Table 1). The observation that gp120 induced a chemotactic response by CD4-bearing lymphocytes, suggests a possible mechanism for accelerating the destruction of uninfected CD4-bearing lymphocytes *in vivo*. Envelope protein shed from viable HIV-1-infected cells⁴¹⁻⁴³ (possibly monocytes/macrophages⁴⁴) might recruit uninfected

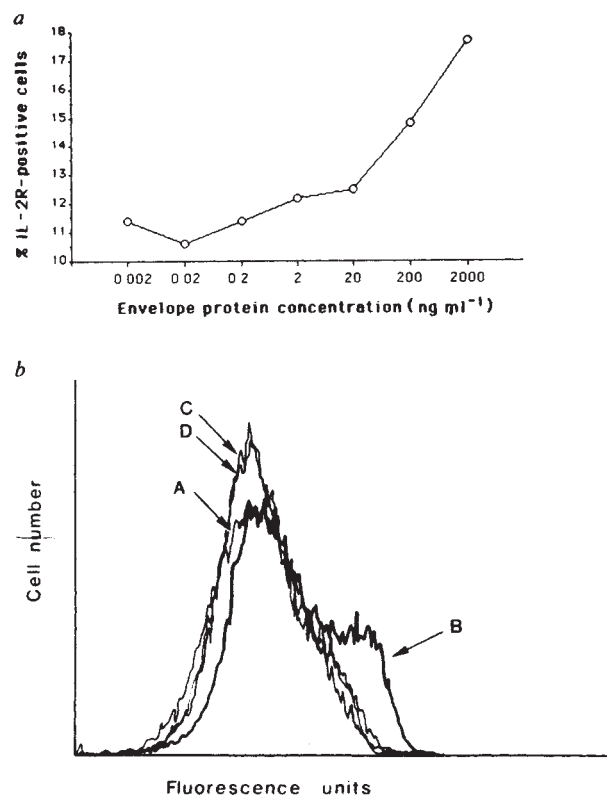


Fig. 3 The HIV-1 envelope glycoprotein induces increased expression of interleukin-2 receptors. *a*, Dose-response of the effect of gp120 on IL-2R expression. T cells were incubated with a range of gp120 dilutions for 24 h then stained with fluorescein-isothiocyanate-conjugated anti-human IL-2 receptor (CD25, Becton-Dickinson) monoclonal antibody and analysed with a Becton-Dickinson FACS 440 as described¹⁰. Less than 5% of cells incubated in medium alone express detectable levels of IL-2R. *b*, Specificity of the effect of gp120 on IL-2R expression. T cells were incubated in control buffer (A), $2.0 \mu\text{g ml}^{-1}$ envelope protein (B), anti-gp120 immunoprecipitated viral supernatant (C), or pre-incubated with OKT4A monoclonal antibody (Ortho) for 1 h and washed before exposure to gp120 (D). After 48 h cells were stained with fluorescein-isothiocyanate-conjugated anti-IL-2R antibody and analysed as above. The shift in IL-2R expression represents an average increase from less than 5% to 26% IL-2R-bearing cells. IL-2R expression in cells incubated with viral supernatant that was immunoprecipitated with anti-gp120 or cells pre-incubated with OKT4A was less than 6%. Immunoprecipitation with OKT8 antibody, or pre-treating cells with OKT4 antibody before gp120 stimulation resulted in 21% and 26% IL-2R-bearing cells respectively.

Table 1 Checkerboard analysis of gp120

		Envelope protein concentration above filter ($\mu\text{g ml}^{-1}$)			
		0	0.2	1.0	2.0
Envelope protein concentration	0	100%	$127 \pm 21\%$	$104 \pm 15\%$	$72 \pm 18\%$
below filter ($\mu\text{g ml}^{-1}$)	0.2	$158 \pm 24\%*$	$131 \pm 18\%$		
	1.0	$208 \pm 19\%*$		$119 \pm 17\%$	
	2.0	$255 \pm 24\%*$			$102 \pm 21\%$

T cells were analysed in the Boyden chamber for motility in response to three dilutions of gp120 added above, below, or above and below the filter as indicated. Results are expressed as percentage $\pm$ standard deviation of migration in control buffer alone. Migration in control buffer was 8.0 ± 1.1 cells per high power field.

* Significant difference ($P < 0.05$) between experimental sample and control by analysis of variance.

CD4-bearing lymphocytes from the circulation to sites where they could be destroyed by syncytium formation or other mechanisms, just as chemoattractant cytokines recruit cells to foci of inflammation.

Our data indicate that binding of gp120 to CD4 activates the IP_3 and calcium signal mechanism in resting T cells, inducing the increased expression of IL-2R and a chemotactic motile response. These results support the hypothesis that CD4 can function as a signal-transducing receptor for a growth factor ligand, and indicate that gp120 can serve as a useful probe for studying normal functions of CD4. A candidate CD4-binding lymphokine, lymphocyte chemoattractant factor induces similar activities in CD4-bearing lymphocytes and monocytes^{10,37,39}.

Cell activation by lectins, phorbol and cytokines have all been found to stimulate virus expression from cells latently infected with HIV-1 (refs 1-3). Although the significance of gp120-induced lymphocyte activation in the pathogenesis of HIV-1

infection remains to be determined, it is possible that signals generated by gp120 binding might activate the target cell to provide a receptive environment for subsequent steps in the infection process or contribute to the expression of cytopathic effects. A common mechanism for this phenomenon could be the induction of cellular transcription factors capable of activating the HIV-1 promoter^{45,46}. Although T-cell activation by envelope protein may be a significant factor in the pathogenesis of HIV-1 infection, there is no evidence for this at present. The implications of innate biological activities of gp120 should be considered in the design and implementation of vaccine studies using this protein.

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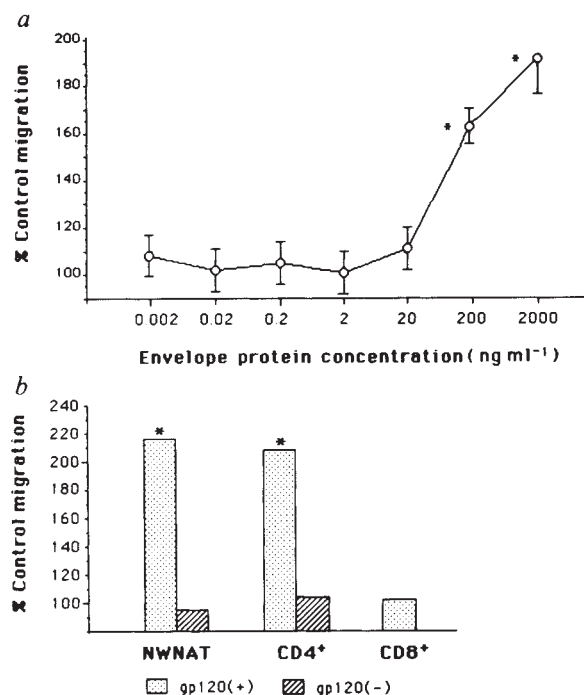


Fig. 4 Chemoattractant activity of HIV-1 envelope glycoprotein *a*, Dose-response of the effect of gp120 on T-lymphocyte motility. T cells were incubated with varying dilutions of gp120 and the motile response was quantitated in a modified Boyden chamber assay³⁹. Results are expressed as a percentage in a modified Boyden chamber assay³⁹. Results are expressed as a percentage of migration of unstimulated control cells $\pm$ standard deviation. *b*, Specificity of the effect of gp120 on lymphocyte motility. Nylon wool-nonadherent T cells, enriched populations of CD4-bearing cells, and enriched populations of CD8-bearing cells were stimulated with $\sim 10^{-8}$ M gp120 and analysed in the Boyden chamber assay. Stippled bars represent the response to gp120 whereas hatched bars represent the response to viral supernatant immunoprecipitated with anti-gp120 serum. *Significant difference ($P < 0.05$) between experimental sample and control by analysis of variance. **Methods.** T cells were suspended in M-199 containing 0.4% bovine serum albumin at 10×10^6 cells ml⁻¹. Enriched populations of CD4-bearing and CD8-bearing cells were prepared by negative selection as described³⁷. This procedure yields cells which are $>95\%$ CD4- or CD8-bearing. Chemotaxis chambers were prepared using 8- μ m pore nitrocellulose filters separating buffer control or experimental samples in the lower wells from cells in the upper chamber. Migration was quantitated by counting total cells moving in the filter beyond a depth of 40 μ m. Migration in buffer control in these experiments ranged from 6.6 ± 1.3 to 10.7 ± 1.5 cells per high power field.

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Structure and function of human perforin

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Perforin (P1) is a cytolytic protein with similarity to complement component C9. P1 has been described as a unique component of murine cytolytic T-cell and rat natural killer cell granules^{1,2}. Previous studies^{3,4} indicated that human granules and P1 differed from murine granules and P1 in that they appeared to be cytolytically less active and lacked the haemolytic activity characteristic of P1. It has been suggested that P1, like C9, is under the control of the homologous restriction factor⁵. Here we determine the primary structure of human P1, re-examine its functional properties, and address the question of homologous restriction.

Figure 1a shows the complementary DNA and derived amino-acid sequence of human P1. Figure 1b shows the homology of human P1 with the human complement proteins forming the transmembrane channel of the membrane-attack complex. The overall homology in the aligned stretch is $\sim 21\%$ for C8 α , 19% for C7 and 17% for C8 β and C9. Only ~ 370 of the 534 amino acids, spanning the putative membrane-binding region and the cysteine-rich epidermal growth factor-type domain, are

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homologous. The amphipathic helix of C9's membrane-binding site⁶ and its distance from the epidermal growth factor-type domain is strongly conserved. Thrombospondin and low-density lipoprotein receptor-type domains are not found in P1. P1 instead contains a length of 40 amino acids at the N-terminal and 130 amino acids at the C-terminal extension of unknown function.

Southern blot analysis of genomic DNA (Fig. 2a) revealed that the coding region of P1 complementary DNA is contained within 4,500 base pairs (bp) of genomic DNA, whereas the gene for C9 exceeds 80 kilobases in length⁷. P1 is encoded in a single locus as a single-copy gene. With the exception of minor expression by HUT 78 cells (a T cell transformed by human T-lymphotropic virus type I) P1 is expressed (Fig. 2b) only by cytolytic lymphocytes. Freshly isolated peripheral blood mononuclear cells (PBL) containing natural killer (NK) cells as the main cytotoxic population, express variable low-to-moderate amounts of P1. Culture of PBL with recombinant interleukin-2 (rIL-2) results in a fivefold increase in the level of P1 messenger RNA within 18 to 30 h (Table 1; Fig. 2b), followed by a decline. The increase of P1 message precedes a 16-fold increase in cytotoxicity by 12 h. High levels of P1 protein appear to be maintained⁸ even when P1 mRNA has decreased to lower steady-state levels.

A puzzling question remained in the finding that human granules isolated from lymphokine-activated killer cells (LAK) cells are haemolytically inactive. To resolve this point, human granules were extracted with high salt and subjected to gel filtration. A protein peak eluted from the column with haemolytic activity in the presence of calcium. After further purification by anion exchange chromatography as described for murine P1 (ref. 9), the active protein migrated on SDS-polyacrylamide gels with a reduced relative molecular mass M_r of 70,000 (70K). It formed membrane lesions characteristic for polyperforin on both rabbit and human erythrocytes (Fig. 3). We also find that haemolytically inactive granules isolated from a subline of a long-term cloned murine cell line, HY3Ag3-M, yield haemolytically active P1 from inactive granules after extraction and gel filtration (see legend to Fig. 3).

Table 1 Induction of cytotoxicity and of perforin expression by rIL-2

Hours of activation	0	6	18	30	44	68
% Cytotoxicity	4	18	40	65	62	73
Relative P1 expression	1	2.5	5.2	4.6	1.4	1.1

Freshly isolated peripheral blood lymphocytes were cultured in $1,000 \text{ U ml}^{-1}$ rIL-2 for various time periods up to 68 h. Cytotoxicity was measured against concanavalin A pulsed ($10 \mu\text{g ml}^{-1}$), ^{51}Cr labelled K562 (10:1 E/T ratio). Expression of P1 relative to peripheral blood lymphocytes equal to unity was determined by quantitative video densitometry of Northern blots.

By analogy with previous experiments on homologous restriction of lysis by C9 (ref. 10), the haemolytic activity of human P1 was compared with that of murine P1 (Fig. 4). Reference for this experiment was provided by rabbit red blood cells (RBC), heterologous to both murine and human P1. Figure 4 shows that RBC are not exceptionally resistant to homologous P1. Human RBC and murine RBC particularly are more resistant to P1 lysis of either species than the rabbit RBC.

The reported findings offer several important insights into the mechanism of cytotoxic lymphocyte-mediated cytotoxicity and its relation to complement-mediated cytotoxicity. The homology comparison of complement proteins with P1 confirms previous predictions on the common genetic origin of the two effector systems¹¹. Neither the low-density lipoprotein receptor-type domain—somewhat surprising in view of the reaction of P1 with an antibody against a synthetic peptide of the low-density lipoprotein receptor-homologous region of C9 (ref. 12)—nor

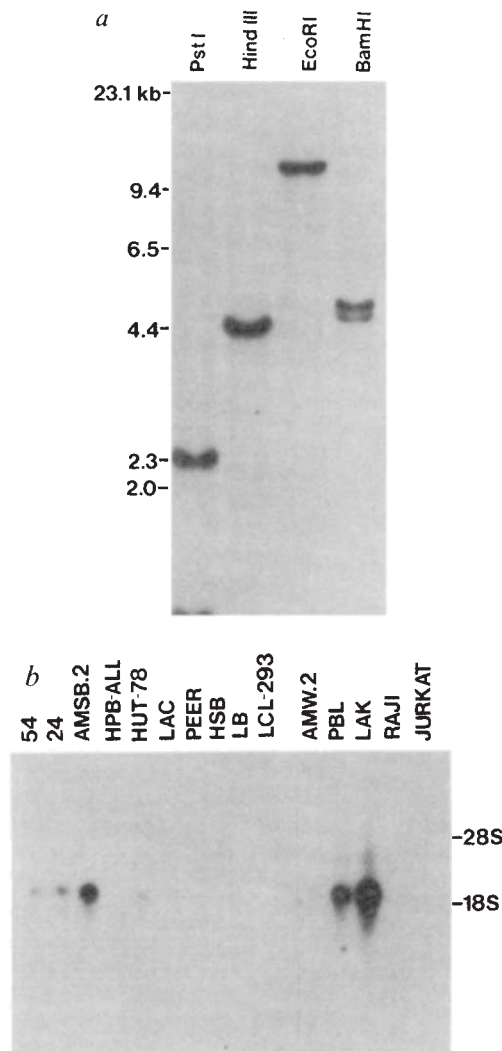


Fig. 2 Southern and Northern blot analysis of human perforin 1. *a*, The perforin gene. Genomic DNA was digested with the enzymes indicated and hybridized with a P1 cDNA restriction fragment, encompassing the coding region plus 90-bp untranslated 3' sequence. *b*, P1 expression in various human cells. CTL clones: 54, 24 and AMB.2. T-helper clone: AMW.2. T-lymphomas: Jurkat, HSB, HPB-ALL HUT-78, LAC, PEER. Epstein-Barr virus transformed B-lymphoblasts: LCL-293, LB, Raji. PBL are freshly isolated peripheral blood lymphocytes. LAK are PBL activated for 48 h in $1,000 \text{ U ml}^{-1}$ rIL-2. The migration of 28S and 18S ribosomal RNAs is indicated. Note that lane 54, 24 LB and AMW.2 only contain 500 ng and all other lanes 4 μg total RNA, as determined by absorbance at 260 nm and ethidium bromide staining.

Methods. Total RNA was isolated according to (ref. 28) and electrophoresed on denaturing formaldehyde containing 1% agarose minigels and transferred to nitrocellulose. After prehybridization, filters were hybridized with random primed ^{32}P -labelled²³ P1 cDNA (1.5×10^6 c.p.m. ml^{-1} in $6 \times \text{SSC}$, $1 \times \text{Denhardt's}$ solution, 0.05% $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$, 0.1% SDS, $100 \mu\text{g ml}^{-1}$ tRNA at 68°C overnight), washed (final wash at 60°C in $0.1 \times \text{SSC}$, 0.1% SDS for 20 min) and exposed to X-ray film with intensifying screen for 2 d at -75°C .

the thrombospondin-type domain is contained in P1. Apparently they are not essential for transmembrane channel formation or for the polymerization reaction. But, in the heteropolymeric assembly of the complement membrane-attack complex¹³, they could allow the association of the different proteins (C6, C7, C8 and C9) it comprises. In addition, these domains could be related to the homologous restriction of lysis by C9, which is not apparent with P1. The isolation of active P1 from haemolytically inactive granules by salt extraction

Fig. 3 Ultrastructure of human poly perforin lesions on human and rabbit red blood cells. Upper panel: poly P1 complexes on rabbit RBC membranes, left arrow indicates complete tubules, right arrows depict incomplete tubules. Lower panels; poly P1 on human RBC membranes. The arrow on the left shows an incomplete circular complex. In the right panel, arrows point to 15 protomers in the poly P1 complex. Scale bar in all panels is 50 nm.

Methods. Isolation and purification of functionally active human P1 was from LAK activated killer cells grown in long-term cultures⁹. The initial fractionation by Percoll was substituted by differential centrifugation. At that point the human granules did not show any haemolytic activity. After extraction with 1 M Na, K, HPO₄ (pH 7.2) Ca-dependent haemolytic activity migrating with apparent M_r 70 K was eluted on Sephacryl 300 (Pharmacia) in isotonic phosphate buffered saline. Pooled active fractions were subjected to ion-exchange chromatography on Mono Q (Pharmacia). Activity was eluted at 0.2 M NaCl in a single fraction. The active protein migrated on SDS-PAGE with a reduced, apparent M_r of 70K. The following activity of granules and perforin before and after high-salt extraction and gel filtration was observed (in z mg⁻¹): human (h) LAK granules 0; Human LAK P1: 750; murine HY3 Ag3-M granules: 0; murine H43 Ag3-M P1: 4,500; CTLL2 granules (positive control): 80; CTLL2-P1: 2187. Zero activity was defined as no detectable haemolysis with 50 μ g granule protein per assay. Haemolysis was determined as described in Fig. 4. The data for CTLL2 were taken from ref. 9. Electron microscopy of rabbit and human RBC membranes lysed with purified P1 in Tris buffered saline, pH 7.4, supplemented with 10 mM CaCl₂. RBC membranes were recovered and treated with 50 μ g ml⁻¹ trypsin and chymotrypsin for 45 min at 37 °C. Membranes were washed several times, resuspended in 10 mM NaHCO₃, pH 7, 0.02% NaN₃ and negatively stained with 2% uranyl acetate.

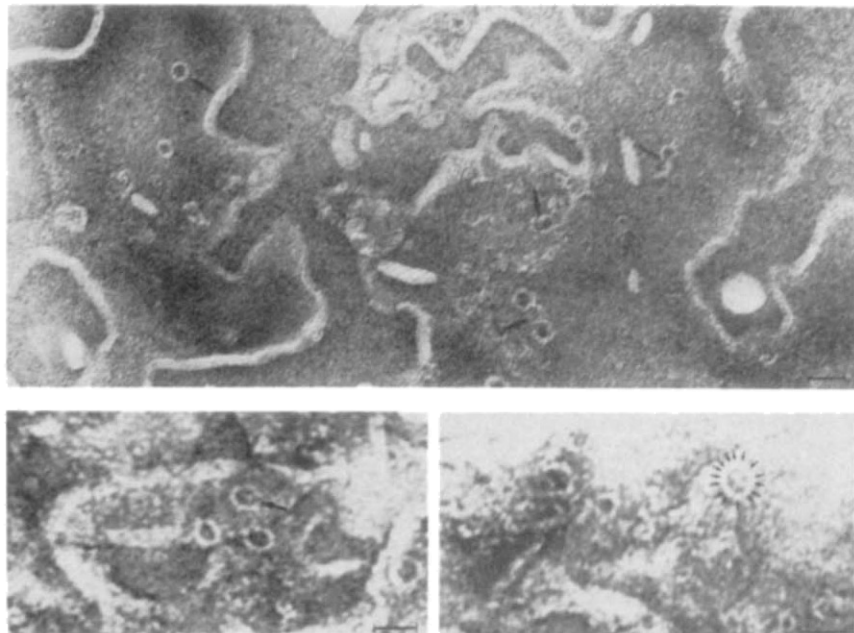
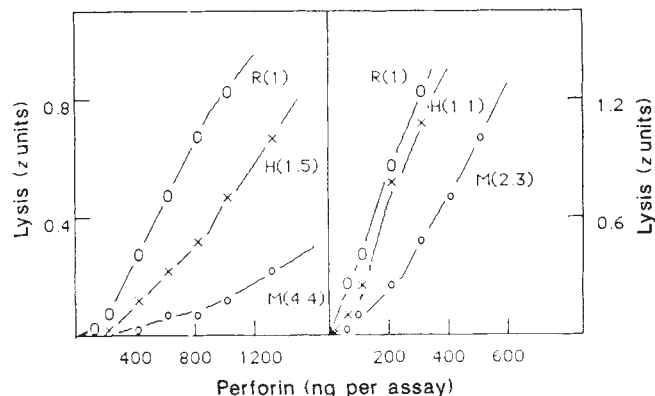


Fig. 4 Apparent lack of homologous restriction of perforin. The haemolytic activity of human P1 (left panel) is compared with that of murine P1 (right panel) using rabbit RBC (large circles), human RBC (crosses) and murine RBC (small circles) as targets. Lysis is expressed in z units by using the Poisson analysis ($z = -\ln[1 - y]$, where y is the fraction of lysed cells). The numbers in brackets are multiplication factors of P1 required to achieve identical lysis to the reference rabbit RBC.

Methods. Human and murine P1 was purified as described in Fig. 3. Haemolytic assays were performed in Tris-buffered saline (pH 7.4) supplemented with 5 mM CaCl₂ at 37 °C for 30 min and haemoglobin release was determined by absorbance at 412 nm. The RBC concentration was calibrated to 1.2 $\times$ the absorbance at 412 nm for 100% lysis.



shows that absence of haemolytic activity in granules cannot be used as an argument to exclude the presence of P1 (ref. 14). It is not clear what factor restricts the activity of P1 contained in granules. The dramatic effect of salt extraction may suggest a role for the highly charged granule proteoglycans.

The availability of cloned P1 cDNA will be essential to resolve the controversial assessment^{15,16} of the importance of P1 and granule secretion in the cytolytic process mediated by cytolytic T cells and natural killer cells.

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Expression of a truncated viral *trans*-activator selectively impedes lytic infection by its cognate virus

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A virion protein of herpes simplex virus type 1 (HSV-1) specifically and potentially activates transcription of the viral immediate early genes. Appropriate function of this protein, termed VP16, depends on an acidic transcriptional activation domain located within the 78 carboxyl-terminal amino acids of the protein. Mutated forms of the protein lacking this acidic domain lose the ability to activate transcription, and can dominantly interfere with the *trans*-activation function of native VP16 (ref. 1). We have prepared stably transformed mouse L cells that constitutively express a form of VP16 lacking its acidic activating domain. In this report we show that these cells are selectively impaired in their capacity to support the lytic infectious cycle of HSV-1, and that this impairment results from their inability to support immediate early transcription.

Mature particles of HSV-1 contain a factor that activates expression of viral immediate early (IE) genes after entering a permissive cell². This IE-activating factor is specified by a phosphoprotein termed VP16 (ref. 3) of relative molecular mass (M_r) 65,000 (65K). Each IE gene contains *cis*-regulatory DNA sequences that mediate response to VP16 (refs 4–7). Recent observations have indicated that VP16 associates with IE *cis*-regulatory DNA sequences during the process of gene activation^{7–9} but, unlike most gene-specific activator proteins that have been studied in bacteria and yeast, VP16 is incapable of independent association with its target genes. The association of VP16 with IE *cis*-regulatory DNA *in vitro* sequences depends on DNA binding proteins endogenous to the host cell^{7–9}. Apparently,

VP16 action is dependent on at least two molecular specificities: protein-protein interaction between VP16 and host cell DNA-binding proteins, and protein-DNA interaction between these host factors and IE *cis*-regulatory DNA sequences.

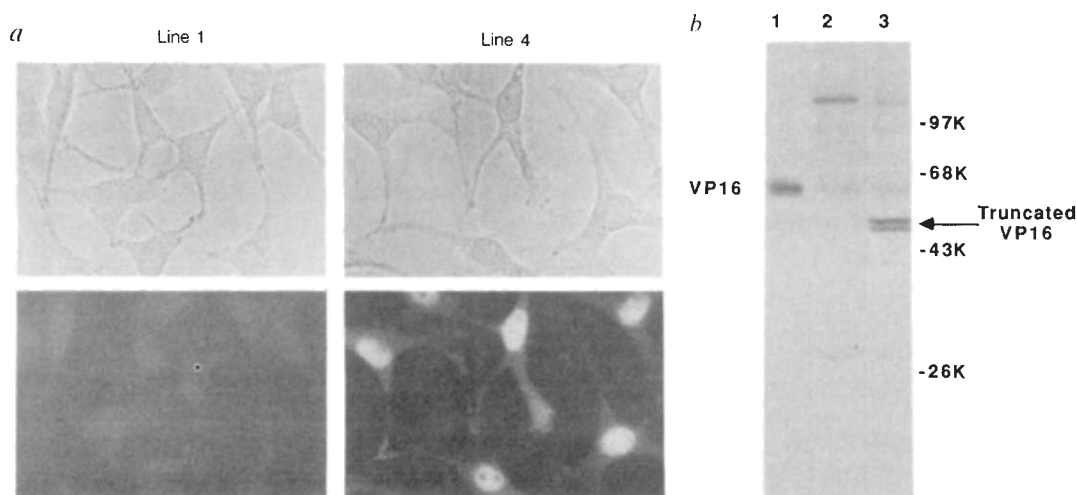
Although VP16 is fundamentally different from prototypical gene activator proteins in its mode of interaction with DNA, once associated with a target gene, it probably activates gene expression by a common mechanism. This conclusion derives from recent studies that identified an acidic transcriptional activating domain with the 78 carboxyl-terminal amino acids of VP16; truncated forms of VP16 lacking this region are incapable of activating IE gene expression¹. Moreover, when this carboxyl tail of VP16 is appended on to the DNA-binding domain of the yeast transcriptional activator protein GAL4 in lieu of the GAL4 acidic domain, it confers potent activation function¹⁰.

Removal of the 78 carboxyl-terminal amino acids of VP16 not only eliminates the activation function, but also creates a form of the protein that dominantly interferes with the native activator¹. The molecular basis for the *trans*-dominance of the tail-truncated form of VP16 is unknown; however, we favour the hypothesis that the truncated protein retains the capacity to interact specifically with the cellular DNA-binding proteins that mediate interaction with IE *cis*-regulatory sequences. As such, truncated molecules might saturate interaction sites on cellular DNA-binding proteins, thereby blocking occupancy of these sites by native VP16 protein. One important aspect of the *trans*-dominance of the tail-truncated form of VP16 is its specificity. Expression of tail-truncated VP16 does not interfere with gene expression directed by *cis*-regulatory elements that are not targets for VP16-specific transcriptional activation.

If the interpretations regarding VP16 outlined thus far are valid, constitutive expression of the tail-truncated form of the activator might be expected to impair the HSV-1 lytic cycle by blocking the natural activator. To test this hypothesis, we prepared stably transformed cell lines that expressed tail-truncated VP16. Mouse L cells deficient in thymidine kinase (tk) enzyme activity were co-transfected with two bacterial plasmids. One plasmid contained a recombinant copy of the HSV-1 *tk* gene, and the other a recombinant copy of the HSV-1 gene encoding

Fig. 1 Expression of tail-truncated VP16 in stably transformed murine L cells. *a*, Indirect immunofluorescent and phase contrast micrographs of cell lines 1 (transfected with HSV-1 *tk* gene alone) and 4 (transfected with the *tk* gene and a truncated form of the HSV-1 gene that encodes VP16). *b*, Western blot analysis of proteins extracted from cell lines 1 and 4.

Methods. For microscopy, cells were grown on acid-etched coverslips in DMEM/10% FCS at 37 °C, 5% CO₂. After washing with PBS, the cells were fixed with 1.6% paraformaldehyde/PBS, permeabilized by treatment with acetone for 2 min at –20 °C, incubated with polyclonal rabbit anti-VP16 (ref. 1), incubated with rhodamine-conjugated, goat anti-rabbit antibody, and visualized under an oil-immersion 63× phase contrast objective using a fluorescence microscope (Zeiss). For Western blot analysis, cells were grown to confluency on 60-mm culture plates, washed with PBS, and lysed in 10-mM Tris-HCl (pH 8.0), 5-mM EDTA, 1% SDS. The lysates were sonicated briefly and precipitated with 4 volumes of acetone. The precipitate was collected by centrifugation, washed with 100% acetone, resuspended in SDS sample buffer, and boiled for 3 min. Samples containing 6 × 10⁵ cell equivalents of protein were electrophoresed on a 12% polyacrylamide SDS gel¹⁴. Equivalence of protein concentration in different samples was verified by Coomassie blue staining (data not shown). Following electrophoresis, proteins were transferred to nitrocellulose¹⁵, probed using polyclonal anti-VP16 antibody (see Fig. 1*a*), and visualized using a second antibody coupled to horseradish peroxidase (Vector Labs). 10% horse serum was included in all antibody and blocking reagents. Lanes correspond to (1) full-length VP16 produced in *Escherichia coli* (C. Vinson and S. McKnight, unpublished results), (2) cell-line-1 protein extract, and (3) cell-line-4 protein extract.



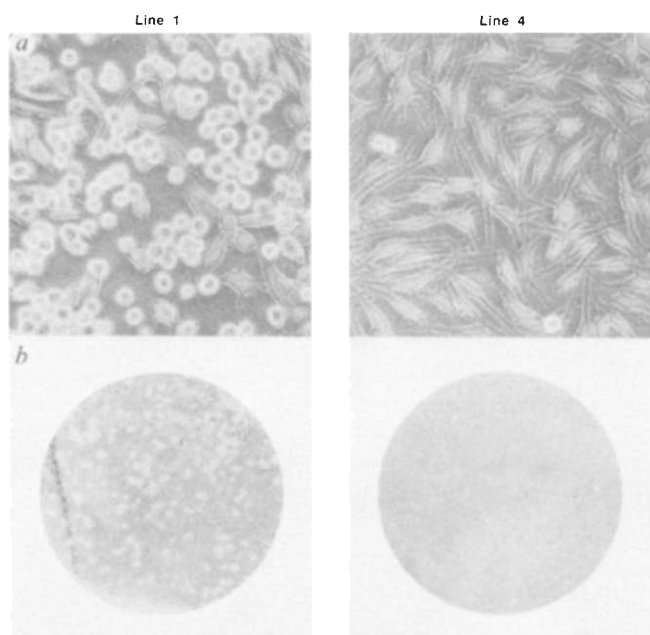


Fig. 2 Comparison of HSV-1 infection of cell lines 1 and 4. *a*, Pathogenic effects of HSV-1 infection on control and experimental cell lines. *b*, Plaque assays of HSV-1 infection on control and experimental cell lines.

Methods. To examine pathogenic effects, subconfluent monolayers of cell lines 1 and 4 were infected with the KOS strain of HSV-1 at a multiplicity of infection of 0.1 plaque forming units (PFU) per cell. After 1-h adsorption at 37 °C, the cells were washed and maintained in DMEM/1% FCS. Living cell monolayers were visualized without further processing by light microscopy 24 h post-infection. For plaque assays, confluent monolayers of cell lines 1 and 4 were infected with 1,000 PFU per culture dish of HSV-1. After 1-h adsorption, cells were washed and covered with 0.95% agarose/DMEM/1% FCS. Three days later the agarose plug was removed and the cells stained with 0.04% crystal violet.

VP16. In the latter case, the promoter of the VP16 gene was replaced by that of the Moloney murine sarcoma virus long terminal repeat, and DNA sequences encoding the carboxyl terminal 78 amino acids of the VP16 polypeptide were deleted. Stable *tk*-positive transformant colonies were identified by selection in HAT (hypoxanthine/aminopterin/thymidine) medium, and 29 clones were examined by indirect immunofluorescence for VP16 expression. Roughly one third of the clones proved positive by this assay, and in all cases the antigenic signal was nuclear. Figure 1*a* compares the immunofluorescence-staining pattern of a VP16-positive transformant (line 4), with that of a line transformed with the HSV-1 *tk* gene alone (line 1). Total cellular proteins from all 29 clones were examined by Western blot analysis and, in agreement with the cytological assay of antibody reactivity, 12 produced the truncated form of VP16 (the same clones that proved positive in the immunofluorescence assay). Figure 1*b* compares Western blot results for lines 1 and 4.

Three tests were performed to examine the growth of HSV-1 on cells that express tail-truncated VP16. First, we simply followed the viral infectious cycle by light microscopy to track pathogenic effects (Fig. 2*a*). Cells that express tail-truncated VP16 (line 4) show minimal evidence of viral pathogenesis under conditions of infection that are uniformly lethal to control cells (line 1). Second, we compared the experimental and control cell lines for their capacity to support formation of viral plaques (Fig. 2*b*). In this assay, line 1 supported plaque formation far more readily than line 4. Third, we monitored virus production in control and experimental cells over a time course of infection. Cells were infected with HSV-1, and culture medium was retrieved at successive intervals post-infection for use in virus

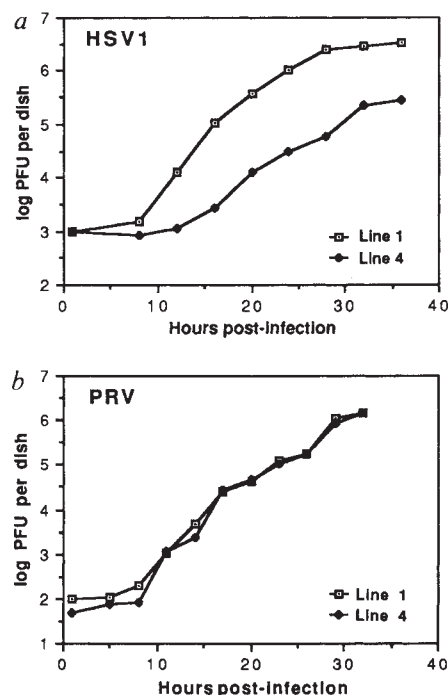


Fig. 3 Kinetics assays of virus growth on cell lines 1 and 4. *a*, HSV-1 growth curves. *b*, PRV growth curves. Symbols: squares, line 1; diamonds, line 4.

Methods. Cell lines 1 and 4 were plated at a subconfluent density on 60-mm culture dishes (1×10^6 cells per dish). The following day, 0.1 PFU per cell of HSV-1 KOS strain or PRV were adsorbed on to each dish. After 1 h the cells were washed twice with DMEM and then maintained in 5 ml of DMEM/1% FCS. Supernatant was removed at 3–4-h intervals. Virus titres of supernatant samples were determined on Vero cell monolayers¹⁶. Each data point represents the average of duplicate samples retrieved from separate infections.

titre assays. The virus growth curves shown in Fig. 3*a* demonstrate a lag in the initial appearance of newly made virus (8–12 h), and a reduction in the amount of virus produced over a time period sufficient for a single lytic infectious cycle (40-fold reduction in virus titre 24 h post-infection). To test the possibility that line 4 is simply defective in releasing virus into the culture medium, duplicate plates of line 1 and line 4 cells were infected as in Fig. 3*a*, and the cell monolayer and culture media were collected together at 24 h post-infection. Intact cells were disrupted by sonication, and the resulting sonicate was titred. Again, a substantial difference in the titre was detected. Similar 20–40-fold impairment of virus growth was detected for an independently isolated cell line that also expresses the truncated form of VP16 (data not shown).

To examine the specificity of resistance of line 4 to HSV-1 we also infected the two cell lines with pseudorabies virus (PRV), and determined the kinetics of virus appearance in the culture medium. PRV was chosen as a control virus because it is very closely related to HSV-1, yet does not encode any IE-activating factor like VP16 (ref. 11). As the data of Fig. 3*b* show, the lytic growth of PRV in line 4 cannot be distinguished from its growth in the control cell line. Moreover, PRV also behaved in the same way on the two cell lines when tested by the plaque assay outlined in Fig. 2*b* (data not shown). As the abrogative effects of expression of the tail-truncated form of VP16 are specific to HSV-1, we doubt that they simply reflect a deleterious effect on cell growth or viability. This possibility was also tested by comparing the doubling times of the experimental and control cell lines. The two lines grew at an indistinguishable rate of one cell doubling every 22 h.

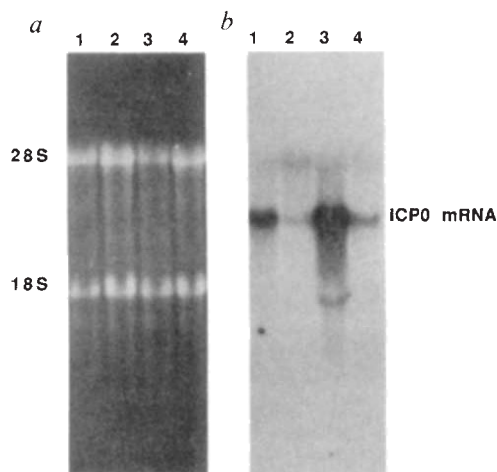


Fig. 4 Analysis of ICPO mRNA synthesized in cell lines 1 and 4. *a*, Ethidium bromide-stained total RNA from cell lines 1 and 4. *b*, Northern blot analysis of ICPO mRNA synthesized in cell lines 1 and 4.

Methods. Confluent monolayers of cells were treated with cycloheximide ($100 \mu\text{g ml}^{-1}$) for 30 min before infection with HSV-1 (KOS strain) at either 0.1 or 0.3 PFU per cell. After adsorption for 1 h, the cells were washed with DMEM and maintained in DMEM/1% FCS/cycloheximide. The 4-h post-infection total RNA was extracted from the cells by the GITC method¹⁷. Samples ($10 \mu\text{g}$) were electrophoresed on a formaldehyde-containing 1% agarose gel¹⁸. The gel was then stained with ethidium bromide to verify mass equivalence and integrity of the various RNA samples. Lanes correspond to: (1) cell line 1 infected at 0.1 PFU per cell, (2) cell line 4 infected at 0.1 PFU per cell, (3) cell line 1 infected at 0.3 PFU per cell, and (4) cell line 4 infected at 0.3 PFU per cell. For Northern blot analysis, $20 \mu\text{g}$ each sample was electrophoresed under the same conditions as in Fig. 4*a* and blot-transferred to nitrocellulose. The filter was prehybridized at 37°C with $5\times\text{SSPE}$ ($1\times\text{SSPE} = 0.18\text{M NaCl}$, $10\text{ mM N}_2\text{H}_4\text{PO}_4$, 1 mM EDTA , $\text{pH } 7.4$), 0.1% SDS, $5\times\text{Denhardt's solution}$, 50% formamide, and hybridized at 37°C in the same solution with a radiolabelled 2.1-kilobase *XhoI-SalI* restriction fragment derived from a recombinant DNA clone of the HSV-1 gene that encodes the ICPO IE polypeptide. After 24-h hybridization, the filter was washed at 60°C with $0.1\times\text{SSPE}/0.1\%$ SDS. Lane order corresponds to that shown in Fig. 4*a*.

The observations outlined thus far indicate that expression of the tail-truncated form of VP16 in a stably transformed cell line selectively impairs lytic growth by HSV-1. The virus growth curves shown in Fig. 3*a* demonstrate a lag in the initial appearance of newly made virus (8–12 h), and a reduction in the amount of virus produced over a time period sufficient for a single lytic infectious cycle (40-fold reduction in virus titre 24 h post-infection). Our interpretation of these observations anticipates a block to IE gene activation in the experimental cell line (line 4). To test whether tail-truncated VP16 impedes activation of IE gene expression during HSV-1 infection, transcription from the IE gene encoding infected cell protein O (ICPO) was monitored by Northern blot analysis. As IE gene products are known to feedback-regulate IE gene expression¹², viral infections were carried out in the presence of cycloheximide. As shown in Fig. 4, the experimental cell line produces far less ICPO messenger RNA than the control line. These observations were quantitated by excising an area of the nitrocellulose filter corresponding to the position of ICPO mRNA in each gel lane, and monitoring radioactive ^{32}P by scintillation spectroscopy. A twelvefold reduction in ICPO expression was quantitated in comparing line 4 with line 1, whether assayed at a multiplicity of infection of either 0.1 or 0.3 PFU per cell.

As *trans*-activators of viral transcription and replication are a feature of animal viruses, our approach may not be limited to HSV. It may be possible to couple the use of *trans*-dominant forms of viral regulatory genes with germ-line transformation

in animals to confer virus resistance directly. Perhaps more importantly, this approach could provide a means of interfering with infection by human immunodeficiency virus (HIV). The HIV genome encodes Tat, a virus-specific *trans*-activator protein. Frankel *et al.*¹³ have shown that Tat forms a stable dimer, and have pointed out that if molecular genetics can be used to generate a *trans*-dominant form of Tat, it may be possible to confer HIV resistance by direct gene transfer.

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Regulation of a wheat promoter by abscisic acid in rice protoplasts

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Although it is well documented that plant tissue responds to phytohormones by expressing new genes^{1,2}, the signal/transduction pathway from hormone to gene is not well understood. DNA sequences regulating plant tissue responses to other external signals such as light³, heat⁴ and anaerobic stress⁵ have been characterized. Regulatory sequences involved in hormonal activation of genes have not been described from any plant, however. We report here the normal regulation of a promoter from wheat, inducible by the phytohormone abscisic acid (ABA), in a transient expression assay using rice protoplasts: an isolated plant promoter fragment is thus responding specifically to hormone treatment, and provides direct evidence for phytohormone acting to regulate the initiation of gene transcription. The response is rapid (within 60 min), which should allow identification of intermediates between the ABA signal and its regulation of gene expression.

Em is a major protein of the mature wheat embryo that begins to accumulate in embryos by 21 days post anthesis⁶, that is, at

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stage III⁶. Embryos removed from the grain at stage II or III and cultured *in vitro* germinate precociously into normal seedlings and Em is not accumulated. If 10^{-6} to 10^{-4} M ABA is present in the culture medium, both the Em messenger RNA (mRNA) and protein are rapidly synthesized and accumulated⁵. Complementary DNA (cDNA) clones of Em, and another ABA-regulated wheat gene (encoding 7S globulin), were used to measure levels and synthesis of these specific mRNAs in mature wheat embryos (stage V) treated with ABA, either alone or in combination with α -amanitin⁷. Results from this study indicated a transcriptional component to the regulation of both ABA-induced wheat genes. The role of ABA in affecting expression of a defined set of genes in other embryo systems is very similar to our results found in wheat⁸. More recently, we have found that transcripts of the Em gene can be accumulated in non-embryonic tissue in response to ABA. Five- to ten-day-old wheat seedlings accumulate Em mRNA only after a 12-h ABA treatment⁹. The rice suspension cultures used in this study (see Fig. 2 legend) also accumulate an Em-like mRNA when incubated in ABA for 16 h (data not shown). Hence, the regulation of Em expression by ABA does not seem to be tissue-specific. Another recently characterized rice gene (RAB21) also appears to be regulated by ABA in a tissue-independent manner¹⁰.

A full-length cDNA of Em¹¹ was used to isolate a genomic clone from wheat to obtain a clearer understanding of the transcriptional control mechanism of ABA induction by identifying *cis*-acting regulatory sequences. Chimaeric genes composed of a translational fusion between 5' flanking fragments from an Em genomic clone and β -glucuronidase (GUS), linked to a 3' fragment from the cauliflower mosaic virus (CaMV) 35S gene were constructed and introduced into rice protoplasts. In this report, we demonstrate that a chimaeric gene with 650 base pairs (bp) of the Em promoter is sufficient for ABA-regulated expression of GUS in a rice transient assay.

The chimaeric genes introduced into rice protoplasts by polyethylene glycol (PEG) treatment are shown in Fig. 1. They differ only in the 5' promoter fragments that are fused to GUS. After uptake of the constructs, rice protoplasts were incubated for 18 h in the presence or absence of ABA and then assayed for

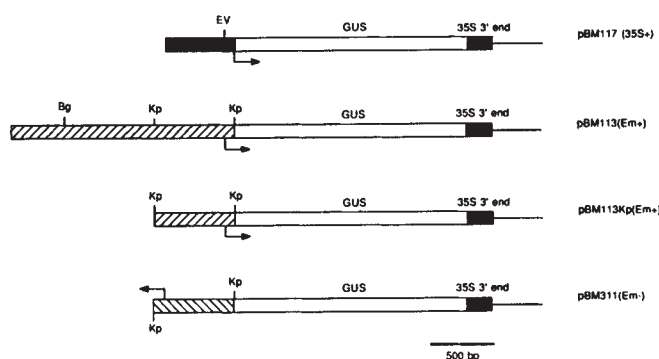
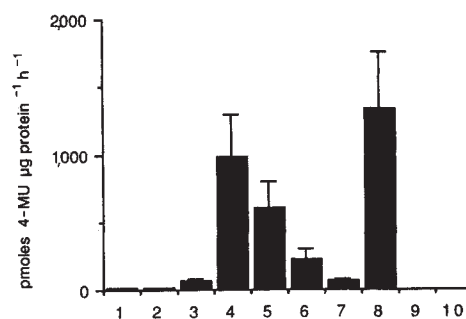


Fig. 1 Construction of chimaeric genes. All plasmids contain the *Escherichia coli uidA* gene flanked 3' by a fragment of the CaMV 35S gene, from coordinates 7,439 to 7,632 (ref. 18). The *uidA* gene encodes a β -glucuronidase enzyme (GUS) and is used as an assayable reporter gene product¹⁹. Plasmid pBM117 contains a CaMV 35S promoter fragment (coordinates 6,909 to 7,437) derived from pDH51 (ref. 18), whereas plasmid pBM113 contains ~1,800 bp of the Em gene from wheat. Plasmid pBM113Kp contains a smaller fragment (650 bp) from the same region of the Em promoter region, and pBM311 contains this same 650 bp fragment in the opposite orientation. Arrows indicate transcription start sites. Abbreviations, EV, *EcoRV*; Bg, *BglII*; Kp, *KpnI*.

Methods. The GUS gene from pRAJ260 (ref. 20) was cloned into pDH51 (ref. 18) as a *PstI* fragment. After deletion of the polylinker sites from *SmaI* to *Sall*, the GUS gene and the CaMV 3' region were cloned into pUC18 as a *KpnI* fragment. This plasmid (pBM109) served as the vector into which all subsequent promoter regions were cloned. The promoter region of pDH51 was cloned into pBM109 as a filled-in *NcoI/SmaI* fragment to yield pBM117. Plasmid pBM117 contains a 40-bp leader sequence, 29 bp derived from polylinker sequences and 11 bp derived from the *uidA* gene. A *Sall/AhaII* fragment of ~1,800 bp from an Em genomic clone (pEAS41.8; J. C. Litts *et al.*, manuscript in preparation) was ligated into pBM109 to yield pBM113. Plasmid pBM113 contains the entire leader region of the Em gene plus 6 bp of the Em-coding region. The 650 bp *KpnI* fragment from pBM113 was cloned into pBM109 and plasmids were isolated which contained the two possible orientations, pBM113Kp and pBM311.

Fig. 2 Analysis of GUS activity in transformed rice protoplasts. Protoplasts were transformed as described below and collected after 18 h incubation. Activity is expressed in pmoles of 4-methyl umbelliferone (4-MU), the product of the GUS enzyme reaction^{19,20}, produced per μ g protein in the protoplast extract per hour of the assay. Columns represent the activity present in the protoplast extract where the input DNA was: pBM117, cultured without ABA (1); pBM117, with 10^{-4} M ABA (2); pBM113, no ABA (3); pBM113, 10^{-4} M ABA (4); pBM113, 10^{-5} M ABA (5); pBM113, 10^{-6} M ABA (6); pBM113Kp, no ABA (7); pBM113Kp, 10^{-4} M ABA (8); pBM311, no ABA (9); pBM311, 10^{-4} M ABA (10). Error bars represent the standard deviation.

Methods. Isolation of protoplasts. Protoplasts were isolated as follows: rice suspension cultures, initiated from callus, were maintained by weekly subculture at a 1:4 dilution ratio with fresh liquid N6 medium²¹, containing 2 mg l⁻¹ 2, 4-dichlorophenoxyacetic acid and 3% (w/v) sucrose, pH 5.8. Protoplasts were isolated from these suspensions 3–7 days after subculture by overnight (16–18 h) enzyme digestion. The digestion was performed by addition of 4 ml enzyme solution (2% w/v cellulase 'Onozuka' RS and 0.5% w/v Macerozyme, both from Yakult Honsha, Nishinomiya, Japan, and 13% w/v mannitol, pH 5.6) per g fresh weight of cells. The mixture was agitated on a rotary shaker at 50 r.p.m. at 25 °C during the overnight incubation. Released protoplasts were filtered through a 60- μ m-mesh nylon screen, and washed twice by centrifugation at 80g for 10 min in Krens' F solution adjusted to pH 5.8 (ref. 22). Protoplasts were purified by resuspending the pellet in N6 medium containing 17% w/v sucrose, centrifuging at 80g for 20 min and collecting the floating layer. Cells were counted with a Fuchs-Rosenthal haemocytometer. To transform protoplasts (2×10^6), they were first centrifuged gently (80g) for 2 min in sterile tubes. The supernatant was removed and discarded, and the tubes were shaken gently to loosen the protoplasts from the bottom of the tube. The DNA sample (1 μ g per 1×10^6 protoplasts), in ≤ 5 μ l of a solution containing 1 mM EDTA/10 mM Tris-HCl (pH 8.0), was added to the protoplasts, followed by addition of 100 μ l PEG solution (40% v/v PEG, 3 mM CaCl₂). The resulting mixture was swirled gently for 10 s, then 1.0 ml of Krens' F solution was added to dilute the PEG. The tubes were incubated on ice for 15–20 min and then centrifuged at 80g for 4 min. The supernatant was removed and the protoplasts were washed once with 1.0 ml of Krens' F solution. The protoplasts were resuspended in protoplast medium (0.2 mM KH₂PO₄, 1 mM KNO₃, 1 mM MgSO₄, 1 μ M KI, 0.1 μ M CuSO₄, 10 mM CaCl₂, 10% mannitol, pH 6.5) at a density of 2×10^6 protoplasts per ml. Each sample was split equally and an equal volume of protoplast medium was added, either containing no ABA or containing twice the desired final concentration of ABA. The samples were incubated at 25 °C in the dark for 18 h. For pBM117, pBM113Kp and pBM311 transformations, 2×10^6 protoplasts were used; for pBM113 transformations 4×10^6 protoplasts were used. All transformations were done in triplicate. For the GUS assay protoplasts were collected by centrifugation at 80g and lysed by the addition of 0.5 ml GUS lysis/assay buffer²⁰. After removal of any solid material from the extract by centrifugation at 14,000g, a portion of the supernatant was used to determine protein content. The remainder of the supernatant was assayed for GUS activity using a fluorogenic assay^{19,20}.



GUS. No GUS activity was detected in rice protoplasts incubated alone or with calf thymus DNA (data not shown).

Expression of GUS driven from the CaMV 35S promoter (pBM117) was the same in the presence or absence of 10^{-4} M ABA (Fig. 2, bars 1 and 2). Under control of the *Em* promoter (pBM113), more than a 30-fold enhancement of GUS activity was observed when ABA (10^{-4} M) was present (Fig. 2, bars 3 and 4). Even in the absence of exogenous ABA, GUS activity from the *Em* promoter was about twofold higher than from the CaMV 35S promoter (Fig. 2, bars 1 and 3). Low levels of endogenous ABA are detected in these protoplasts and could account for this activity. Expression of GUS in rice protoplasts was proportional to ABA concentration from 10^{-4} to 10^{-6} M (Fig. 2, bars 4–6, respectively), which is identical to the range of ABA concentrations that inhibit precocious germination of wheat embryos (data not shown). Deletion of the *Em* promoter to 650 bp (pBM113Kp) resulted in an increase in GUS activity

over the 1,800 bp promoter (pBM113) (Fig. 2, bars 4 and 8). This could be indicative of a silencer element in the *Em* promoter between $-1,800$ and -650 , similar to the results reported for the pea *rbcs-3A* gene¹². No GUS activity was detected in the presence or absence of ABA when the orientation of the 650 bp *Em* promoter was reversed (Fig. 2, bars 9 and 10). In addition, a time-course experiment demonstrated that ABA-inducible expression of GUS activity from the *Em* promoter was clearly detectable at one hour, possibly earlier (Fig. 3).

RNase protection experiments were done to determine if the normal wheat transcription-initiation site was used in transient expression of pBM113Kp in rice protoplasts, and if newly initiated transcripts were found only in cells treated with ABA. RNA was extracted from five sources (Fig. 4): *in vitro* cultured wheat embryos treated with ABA (lane 1); rice protoplasts containing an *Em* genomic fragment incubated without (lane 2) and with (lane 3) ABA; and, rice protoplasts containing the

Fig. 3 Time course of ABA response. Protoplasts were transformed with pBM113Kp plasmid DNA. ABA was added, and at various times, aliquots were removed and assayed for GUS activity. Solid bars represent activity from pBM113Kp-transformed protoplasts cultured without ABA; stippled bars represent pBM113Kp-transformed protoplasts cultured with ABA. Insert is an expansion of zero and 1 hour time points. Activity expressed as in Fig. 2. **Methods.** Protoplasts (2×10^6) were isolated and transformed in triplicate as described in Fig. 2 legend. After incubation for 2.5 h in protoplast medium, ABA was added to a final concentration of 10^{-4} M. An aliquot was immediately removed (time 0) for analysis of GUS activity and similar aliquots were removed at 1 h, 2 and 6 h. Individual aliquots were collected, lysed by addition of 0.5 ml GUS lysis/assay buffer and stored at -70°C . After all aliquots were taken, GUS activity was assayed^{19,20}.

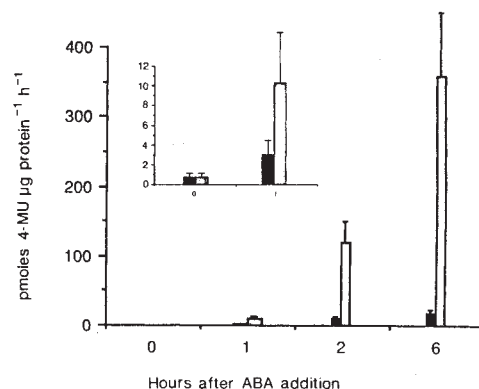
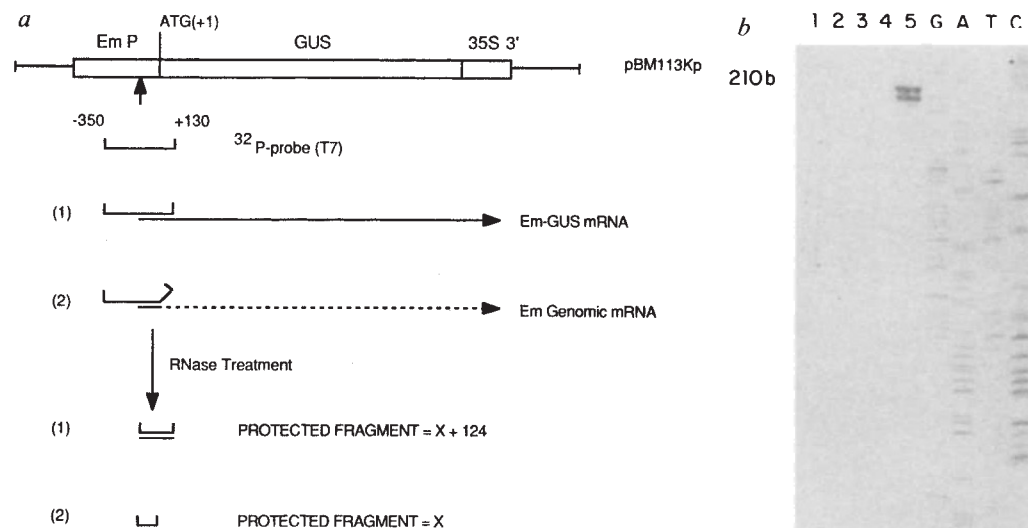


Fig. 4 Analysis of RNA from transformed protoplasts. **a**, RNase protection rationale. Total RNA was isolated from protoplasts as described below and annealed to a ^{32}P -labelled T7 RNA polymerase-generated probe derived from pBM113Kp. The probe represents sequences from -348 bp to $+130$ bp beyond the translational start of *Em*. This probe was annealed to RNA isolated from stage III wheat embryos cultured in the presence of ABA (2) and to RNA isolated from protoplasts transformed with pBM113Kp plasmid DNA (1) or with an *Em* genomic clone (2). The



annealed RNA was then subjected to RNase digestion to remove all single-stranded RNA; double-stranded RNA was protected from digestion. The samples were denatured and separated on a sequencing gel to determine the sizes of the protected fragments. The fragment protected in RNA samples derived from stage III + ABA embryos or from protoplasts transformed with the *Em* genomic clone (2) would be of an unknown size because the transcription start site had not been previously determined. In addition, these protected fragments should be of the same size if transcription was initiated correctly in the protoplast system. The fragment protected in RNA samples from protoplasts transformed with pBM113Kp (1) would be expected to contain all those same sequences plus the sequences representing homology with the remainder of the probe (coding region/GUS). The increase in the sizes of the protected fragments from pBM113Kp-transformed protoplasts would be predicted to be 124 bases because of the presence of the two *Em*-derived amino-acid codons in pBM113Kp. **b**, Autoradiograph of RNase protection gel. Samples are: lane 1, RNA isolated from stage III + ABA wheat embryos; lanes 2 and 3, RNA from protoplasts transformed with an *Em* genomic clone (lane 2, no ABA; lane 3, 10^{-4} M ABA); lanes 4 and 5, RNA isolated from protoplasts transformed with plasmid pBM113Kp (lane 4, no ABA; lane 5, 10^{-4} M ABA). Lanes G, A, T and C represent sequencing reactions used on the gel as size markers. The uppermost bands of the protected fragments are as marked.

Methods. Total RNA was isolated from transformed rice protoplasts²³ and resuspended in diethylpyrocarbonate-treated H_2O . ^{32}P -labelled RNA was generated from a pBM113Kp-derived *Bcl*I/*Sal*I fragment cloned into *Bam*HI/*Sal*I-cut pGEM-3Z (Promega-Biotec) following the manufacturer's protocol. The labelled RNA was annealed to the unlabelled rice RNA at 42°C overnight and the samples were then digested with RNase at 37°C for 1 h (Promega-Biotec protocol). The samples were separated on a 6% acrylamide, 7M urea sequencing gel. The gel was dried onto Whatman 3-MM paper and autoradiographed overnight.

Em-GUS construct (pBM113Kp) incubated without (lane 4) and with (lane 5) ABA. The extracted RNA was annealed to an RNA probe that spanned the translation start codon of the native *Em* gene (Fig. 4a). RNA protected fragments were found only in ABA-treated samples (Fig. 4b, lanes 1, 3, 5). The same transcription initiation site was used in both wheat embryos and rice protoplasts treated with ABA (that is, 86 bp 5' to the translational start ATG codon). The same result was obtained with the native *Em* gene or with the *GUS* gene fused to the *Em* promoter (Fig. 4). These data clearly indicated that at least a part of the ABA control resided at the transcriptional level and that the normal transcription start site of the wheat *Em* promoter was correctly recognized in rice protoplasts, irrespective of the coding sequences downstream. But these results do not exclude an effect of ABA at a post-transcriptional level (such as mRNA stability), which has been shown to account for another part of the ABA control of *Em* mRNA levels *in vivo*⁷. Experiments using transient assay are under way to elucidate such controls.

Analysis of stable chimaeric genes transformed into plants has been the primary approach to characterize *cis*-acting sequences involved in gene regulation. In the case of most cereals, however, the routine use of transgenic plants for analysis of cereal genes in homologous systems is not yet possible. Consequently, protoplasts have been extensively used to introduce and characterize cereal genes using transient assays¹³. In the case of the anaerobic regulation of the maize alcohol dehydrogenase (*Adh1*) gene, both transient assays of *Adh1* expression in maize protoplasts¹⁴, and stable transformation in a heterologous tobacco system⁴ identified similar regions of the 5' flanking sequences that are both necessary and sufficient for induction of *Adh1* expression by low oxygen concentrations. Hence, transient expression assays can be valuable in identifying regulatory sequences in genes from plants. In addition, protoplasts from barley aleurone cells¹⁵ and parsley suspension cultures¹⁶ respond in a normal physiological and molecular manner to the signals of gibberellic acid and a fungal elicitor/ultraviolet light, respectively.

Our experiments demonstrate the expression and normal regulation of an ABA-inducible promoter from wheat when it is incorporated into rice protoplasts. The *Em* promoter (650 bp) without its normal coding and 3' regions, is sufficient for ABA induction of the reporter gene. The ABA response of the *Em* promoter expressed in rice protoplasts is similar to the ABA regulation of the natural gene in wheat embryos, both with respect to the dose-response levels of ABA and the initiation of RNA transcripts. We are now in the process of using the rice transient system to further analyse the particular sequences responsible for ABA regulation in both the *Em* promoter and in promoters of other ABA-regulated genes. Constructs will be stably introduced into fertile plants of tobacco and other dicots to analyse tissue-specific and ABA-regulatory properties of the promoters. The successful recognition of the regulatory factors necessary for the ABA control of wheat promoters in rice protoplasts suggests that regenerated rice plants¹⁷ may be used in the future to characterize regulatory sequences of wheat and other cereal genes that have been introduced into rice.

We thank James C. Litts for the wheat *Em* genomic clone (pEAS 41.8), Roswitha Hopkins (Oregon State University) for wheat embryo RNA, Roy Chaleff and Richard Jefferson for help with rice tissue culture and the GUS assays and constructs, and Sonja Schmitz for technical assistance.

Note added in proof: J. Gómez *et al.*²⁴ reported an increase in mRNA, encoding a protein of relative molecular mass 15,000 rich in glycine, in response to ABA or desiccation. Recently, the role of ABA in water-stressed plants has been reported²⁵.

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Phosphonate biosynthesis: isolation of the enzyme responsible for the formation of a carbon-phosphorus bond

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The first isolation of a naturally occurring phosphonate in 1959¹ led rapidly to the discovery of a variety of metabolites containing a phosphorus-carbon bond². Phosphonates have been found in bacteria, fungi, and higher organisms such as the snail schistosome vector *Biomphalaria*²⁻⁶. The biosynthetic path to the P-C bond has, however, remained undefined. Thus although it was shown twenty years ago that the isotope label from [¹⁴C]glucose^{7,8} or from [³²P]phosphoenolpyruvate⁸ is incorporated into 2-aminoethylphosphonate by the protozoan *Tetrahymena pyriformis*, the presumed stoichiometric transformation of phosphoenolpyruvate to phosphonopyruvate has never been demonstrated. Low conversions of phosphoenolpyruvate into 2-aminoethylphosphonate and the trapping of phosphonopyruvate from phosphoenolpyruvate have been reported⁹⁻¹², but these reactions have not proved reproducible, and the existence of the critical enzyme, phosphoenolpyruvate phosphonmutase, has remained notional. We now report experiments that resolve this enigma, and describe the isolation and characterization of the pure mutase from *T. pyriformis*.

Previous work aimed at detecting the phosphoenolpyruvate phosphonmutase focused on P-C bond formation, and was centred on the conversion of phosphoenolpyruvate to phosphonopyruvate.



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These studies were constrained by the lack of a convenient assay for phosphonopyruvate, and we opted to follow the putative mutase reaction in the opposite direction, with phosphonopyruvate as the substrate and using pyruvate kinase/ADP with lactate dehydrogenase/NADH to monitor the formation of phosphoenolpyruvate. This assay has enabled the identity of the mutase to be established, and, as foreshadowed by recent metabolic experiments with *Tetrahymena*¹³, leads to the conclusion that the equilibrium between phosphoenolpyruvate and phosphonopyruvate lies predominantly towards phosphoenolpyruvate.

The finding of an effective assay for the phosphoenolpyruvate mutase has allowed us to isolate this enzyme. The purification of the mutase from *Tetrahymena* is summarized in Table 1, and yields homogeneous protein having a specific catalytic activity of 74 unit mg⁻¹. When phosphonopyruvate¹⁴ is added to a solution containing purified enzyme, the conversion to phosphoenolpyruvate is quantitative, as monitored by ³¹P NMR and by enzyme assay. The overall equilibrium constant is >100 in favour of phosphoenolpyruvate. The steady-state kinetic parameters in 100 mM triethanolamine-HCl buffer containing MgSO₄ (8 mM) at pH 7.7 and 30 °C, are: k_{cat} 41 s⁻¹, and K_m 67 μ M, and k_{cat}/K_m 6×10^5 M⁻¹ s⁻¹. The enzyme is inactive in the presence of EDTA, but full catalytic activity is regained on the addition of divalent magnesium (in excess over EDTA).

Table 1 Purification of phosphoenolpyruvate phosphomutase

	Total protein* (mg)	Total units†	Specific catalytic activity (units mg ⁻¹)	Purification (fold)
Crude	995	152	0.15	1
DEAE-cellulose	30	62	2.1	14
Hydroxylapatite	0.24	18	74	490

* Protein content in mg ml⁻¹ = $1.45 A_{280nm} - 0.74 A_{260nm}$, where A is absorbance.

† One unit is that amount of enzyme required to process 1 μ mol of phosphonopyruvate per min at 30 °C under the conditions described in the text. The relatively low yield derives not from protein instability, but rather from the sacrifice of units for purity.

From denaturing gel electrophoresis, the protomer relative molecular mass (M_r) is 33,000 (33K), and from gel filtration experiments under non-denaturing conditions, the M_r is 69,000. The enzyme is evidently an α_2 dimer. The N-terminal sequence of the first 30 residues of the mutase has been determined as S-S-T-R-K-T-T-Q-L-K-N-M-I-Q-S-K-D-L-S-F-I-M-E-A-H-N-G-L-S-A.

When phosphonopyruvate is added to a cell-free extract from the snail *Biomphalaria glabrata* or from the protozoan *T. pyriformis*, the fate of the phosphorus can readily be tracked by ³¹P NMR. The results obtained using a *Tetrahymena* extract are illustrated in Fig. 1. Phosphonopyruvate is rapidly consumed, with the initial formation of phosphoenolpyruvate. The phosphoenolpyruvate (which was identified both by additions of authentic material and by coupled enzyme assay) was subsequently transformed into 2- and then 3-phosphoglycerate and inorganic phosphate (P_i) (see Fig. 1), presumably by the action of endogenous enolase, phosphoglycerate mutase and phosphatase(s) in this crude extract. A small amount of a new phosphonate (having a ³¹P resonance at 17.4 p.p.m.), probably 2-aminoethylphosphonate, is also formed. In the case of the *B. glabrata* extract, most of the added phosphonopyruvate was converted into P_i. Although the existence of a phosphonate in this extract has not been established, the result is consistent with the initial isomerization of phosphonopyruvate to phosphoenolpyruvate catalysed by the mutase, followed by the rapid hydrolysis of this phosphate monoester (or of others, such as

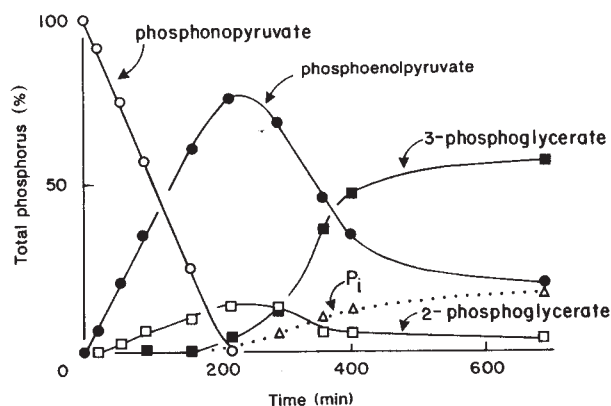


Fig. 1 Fate of phosphorus when phosphonopyruvate is added to a cell-free extract from *T. pyriformis*. Phosphorus is tracked by ³¹P NMR.

2- and 3-phosphoglycerate, derived from it). This latter interpretation is strengthened by the fact that inclusion of the coupling enzymes for the phosphoenolpyruvate assay resulted in the expected changes in NADH, consistent with the trapping of first-formed phosphoenolpyruvate.

Although the earlier claims to have detected the mutase by the formation of equilibrium amounts of phosphonopyruvate from phosphoenolpyruvate must have been mistaken, and although the *in vivo* formation from phosphoenolpyruvate of metabolites that contain P-C bonds requires some strongly exergonic reaction of the first-formed phosphonopyruvate (decarboxylation or transamination?), the now demonstrable existence of the mutase is consistent with much of what has been reported on the biosynthesis of phosphonates. The extent to which the mutase will turn out mechanistically to resemble such enzymes as phosphoenolpyruvate carboxylase, pyruvate kinase or chorismate mutase (for each of which the distal bond to the ether oxygen of enolpyruvate is broken, and a new bond to C-3 of this moiety is made) remains to be seen. The *in vivo* fate of phosphonopyruvate is, moreover, uncertain, as is the pathway by which phosphinates^{5,6} are derived from this first phosphonate. But the isolation of phosphoenolpyruvate mutase now opens the way to the resolution of these and other questions.

We thank Professor D. Dunaway-Mariano for informing us about her independent experiments, to Professor K. Carr and Jack Quinn for advice and help, and Dr W. Lane for the protein sequencing. This work was supported by the National Institutes of Health, the National Science Foundation, and the SERC.

Note added in proof: A partial purification of the phosphonmutase has recently been described by Bowman *et al.* (*J. Am. Chem. Soc.* **110**, 5575-5576 (1988)). The results obtained by these workers are in broad agreement with those reported here.

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Key work for the chemist

W. Graham Richards & Jane M. Burridge

Tetrahedron Computer Methodology. Editor-in-chief W. Todd Wipke. Pergamon. 6/yr. £480, \$750 (institutional); individual rates also available*.

IT USED to be possible to raise a cheap laugh by saying that running a computer program no more made one into a theoretical chemist than sleeping in a garage turned one into a motor mechanic. Nevertheless, over the dead bodies of the old guard, computers are taking over much of chemistry. In job advertisements the terms 'computational chemist' and 'molecular modeller' are now to be seen. Since the early 1960s the impact of computational work has grown — as has its accuracy and realism — in a direct relationship with the development of computer hardware.

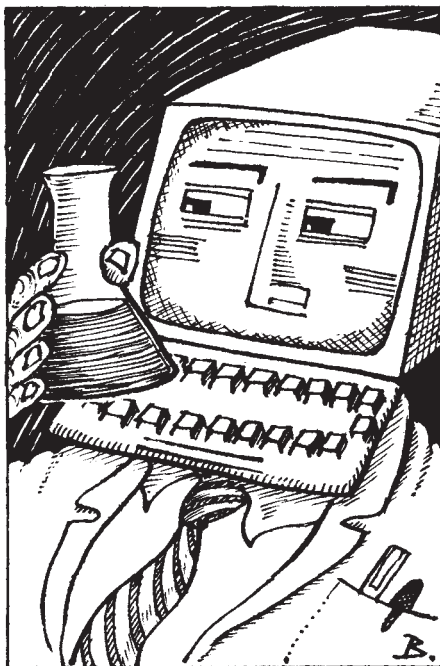
In the area of theoretical calculations there has been rapid progress from the era of crude semi-quantitative results (marginally useful in interpreting experiments), to calculations which match experimental accuracy for small gas-phase molecules. Calculations that incorporate both dynamic effects and the all-important influence of solvent water are now routinely run for macromolecules. The recent work using free-energy perturbation methods represents a new high point.

The extension to larger molecular systems has involved deploying the latest computer graphics devices, more often seen in flight simulators, so that molecules may be displayed, explored and the results of both computation and structure determination portrayed. The explosion of this type of research, and in particular its spread into the pharmaceutical and agrochemical industries, has created a demand for the publishing of colour pictures and also machine-readable programs and data; these needs have been partially met by new journals, and we are beginning to see them addressed by new styles of publication.

Chemistry, and particularly organic chemistry, is an obvious discipline in which to start electronic publishing. The field is overflowing with masses of information on the millions of known compounds. Computer programs are used to search databases, in designing syntheses, in understanding molecular properties and in predicting reactivities. It is into this arena that the executives of the board of editors of *Tetrahedron* have launched *Tetrahedron Computer Methodology*.

The new journal (subtitled *The International Electronic Journal for Rapid Publication of Original Research in*

Computer Chemistry) is expected by the editors to accelerate developments in the field — improving speed and quality of publication by using electronic methods throughout; making data and programs available to subscribers in a directly usable form; and facilitating the publication of new kinds of research work. The journal



will concentrate on new methods for handling chemical information, representations, algorithms and methods for solving chemical problems, particularly those related to organic chemistry. Topics to be covered range from artificial intelligence applications to structure elucidation (presumably by calculation) and the preparation of scientific documents.

The first number, which appeared this summer, consists of the printed journal, four diskettes and a glossy brochure, all in a neat ring binder. Nowhere in the packaging or contents could we find a definite statement of the intended hardware, or indeed any instructions; however there are various hints that at least the Apple Macintosh and IBM-PC (and compatibles) are target machines. We successfully read the 5.25" diskettes from the review copy under DOS on both IBM-PC AT and PS/2 machines. The four diskettes held the full ASCII text (with mark-up tags) of all the journal articles; *ChemText* document files for three of the articles; executable (and in one case source) code,

sample data, documentation and so on for the three programs described in the journal; and various tools for use in *ChemText* document preparation for submission of papers.

Having the full ASCII text on diskette is less useful than one might at first suppose. Loading a file into one's favourite editor allows text search, but the lack of formatting and figures, and the presence of the mark-up tags, are all rather irritating. It would not be difficult to adapt an existing editor or formatter to accept the tags; given that the printed journal is available, however, then for reasonably short articles such as these it hardly seems worthwhile doing other than reading the conventional text. An ASCII version, though, might be a good way to present long and discursive texts — instead of, rather than as well as, the printed version.

For subscribers with access to the *ChemText* software, the provision of *ChemText* document files of journal articles has definite advantages. *ChemText*, from Molecular Design Ltd, is an image and text processor designed specifically for chemistry (see the review in *Nature* 333, 610; 1988). It allows the creation of documents containing, *inter alia*, text, complex molecular representations, mathematical notation, reaction schemes, together with data and graphics retrieved from Molecular Design's — and other — software. In the first issue, readers may view the three articles provided as *ChemText* document files, properly formatted and with integrated graphics. In addition, components of the papers (bibliographies, figures, reactions) may be extracted and filed separately or used as input for further computation.

The programs in this issue cover depth-shaded printing of 3-D structures on a laser printer; exploration of the scope of an intramolecular reaction; and automatic reference and bibliography formatting. The printing program worked as per instructions, but there were no screen graphics to monitor the current orientation as angles were varied. We were forced to check by producing hard copy — no particular problem if the laser printer is attached to one's own machine but a source of potential irritation to users having to go further than the next office. The reaction program caught us out briefly until we realized that instruction entry was case-sensitive; thereafter it worked well in CGA, but not in EGA, display mode. All we got in EGA mode was an error message in French. The bibliography formatting system is a very sound idea, in that once a reference is correctly defined, all future references to it by users with access to the definitions are via unique tags. All rules for reference- and bibliography-handling require a certain discipline on the part of the

* Reduced rates for Vol. 1 (1988) plus Vol. 2 (1989), a total of nine issues.

authors, but this has great potential in facilitating accurate and unambiguous referencing.

It is clearly in the publisher's interest to encourage the spread of software developed by their recent acquisition, Molecular Design. But the heavy emphasis on *ChemText* in this journal seems rather at odds with its stated aim of bringing information in directly useful forms within reach of research chemists. By no means all chemists have access to *ChemText* or other Molecular Design software. Would-be subscribers to the journal who do not are faced with the choice of either making the fairly substantial financial investment involved, or being second-class readers with no facility for viewing text and graphics electronically, and with penalties, both in terms of publication time and inclusion of figures and complex notation, if they submit papers in other than *ChemText* format — even if the submission is in electronic form.

One might expect a true electronic journal to be self contained, in the sense that it should include the software to format text and graphics to the user's screen, and to search for and extract pieces of information for use elsewhere, as well as providing directly machine-readable data, code and so on. *Tetrahedron Computer Methodology* could move in this direction by including *ChemText* as part of the publication, presenting all submissions as *ChemText* document files and dispensing with the printed and ASCII texts entirely. In its present form, however, the journal is perhaps better considered as one with a machine-readable component, and as such we think it is the first to package diskettes with the printed issue. However, in concept it was piped to the post by at least one journal — the *Journal of Computer-Aided Molecular Design* (reviewed on p.462 of this issue) has for some time been making molecular-modelling information from its articles available in machine-readable form.

There is clearly a great need in chemistry for mechanisms to speed the publication process and to distribute information of all types to workers in the field. *Tetrahedron Computer Methodology* is a timely step forward with its electronic submission, refereeing and hard-copy preparation, and its provision of machine-readable data. But a true electronic journal it is not — although computers may have taken over much of chemistry, more imagination is required before they can be said to have taken over chemistry publishing. □

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New journals June 1986 to May 1987

CRITERIA for journals to be considered for review in this issue were circulated to publishers earlier this year, and were also published in *Nature*. They were that:

- (i) the first number appeared, or the journal was re-titled, between June 1986 and May 1987 (although journals not covered in last year's review issue were also considered)*;
- (ii) the journal is published at least three times a year;
- (iii) the main language used is English;
- (iv) where possible at least four issues should be made available for review, including the first and the most recent numbers.

Last year's journals review supplement covered publications appearing between June 1985 and May 1986 and the second cut-off date, May 1987, allows for enough issues of a journal to have been published for a reasonable sample to be available for judgement (many are quarterlies). A spread of four different issues is taken as the usual minimum on which reviewers' comments can be based.

Exceptions have been made to these rules — *Tetrahedron Computer Methodology* (reviewed on the previous page) was launched this summer, but its electronic component makes it of unusual interest; and although there are only two issues annually of *Reviews of Magnetic Resonance*

in *Medicine* (see p.477), this journal complements another one reviewed here three years ago.

After two 12-month periods in which the flow of new journals seemed to slacken, June 1986 to May 1987 provided a deluge of them. A large number of titles submitted for review are not covered in the following pages, but they are listed on p.478.

The brief given to reviewers was to limit themselves to comment on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample of issues, and apply to mid-1988 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of material submitted for review.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1989. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rates with the publisher concerned. □

*See *Nature* 329, 357-376 (1987). For previous journals review supplements see *Nature* 323, 359-379 (1986); 317, 293-308 (1985); 311, 309-330 (1984); 305, 477-497 (1983); 299, 491-514 (1982); and 293, 341-369 (1981).

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Building Bacon's new engine

Donald Michie

Applied Artificial Intelligence. Edited by Robert Trappl. *Hemisphere*. 4/yr. UK £77, elsewhere \$135.

Artificial Intelligence Review. Edited by Masoud Yazdani. *Blackwell Scientific*. 4/yr. UK £48, North America \$99.50, elsewhere £58 (institutional); UK £15.50, North America \$32, elsewhere £18.50 (personal).

The Knowledge Engineering Review. Edited by John Fox. *Cambridge University Press*. 4/yr. UK £53, North America \$105, elsewhere £56 (institutional); UK and elsewhere £32, North America \$52 (personal).

Machine Learning. Edited by Jaime G. Carbonell, Ryszard S. Michalski and Tom M. Mitchell. *Kluwer*. 4/yr. Dfl 256, \$128 (institutional); Dfl 120, \$45 (personal).

ONE of the marks of a new-born discipline is a need for proverbs. In machine intelligence, John McCarthy, who originated both the LISP programming language and the situational calculus, seems also to be the source of many community saws. I have always liked his parable of AI's own 'publish or perish' syndrome. The eager worker writes a computer program. Having elicited some cute behaviours he calls in his friends to watch, and sends a commentary to the nearest journal or proceedings. It is now someone else's turn to announce 'Look Ma, no hands!'.

When the supporters of each new aspirant press the claim that theirs is a discipline rather than a cult, the science club is rarely in any particular hurry to open its doors. What exactly are these newcomers collectively seeking to discover or demonstrate? Can they design a controlled experiment? Do they document what they do so that others can attempt repetition? Do they know about priorities, bibliographies, acknowledgements and the other niceties?

The race of editors, to whom falls the duty somehow to constrain the would-be recruits into shapes of acceptability, bears a special burden in the field of AI — bewitched from birth by its own consequentiality, yet with devotees drawn from backgrounds mostly devoid of contact with experimental method. Of four journals making their debut during the period covered by this supplement, only *Machine Learning* addresses this classic editorial role as maker and shaper of standards. The other three, although printed and presented in journal style, are directed to other functions. I will discuss them first.

Applied Artificial Intelligence is in essence a high-level newsletter for practitioners, "intended to be used", says the launch editorial, "as a means of exchanging information about advances and experiences in the artificial intelligence (AI) field". The more 'surface' style intrinsic to the newsletter genre allows the editor also to claim with justice that it will "aid decision makers in industry and management in understanding the accomplishments and limitations of state-

of-the-art AI". Robert Trappl, who directs Vienna's AI Institute, presides over a collective of some 25 associate editors and board members. Their range of national affiliations is in contrast to the mid-Atlantic emphasis of AI's earlier days. Along with Austria (5), the United States (5), Japan (3) and Italy (2) we find one representative each from Belgium, Britain, Czechoslovakia, France, Poland, Portugal, the Soviet Union, Sweden,

Machine Learning

Switzerland and West Germany. The publication thus forms a long-needed complement to the American Association for Artificial Intelligence's *AI Magazine*.

Twenty papers appeared in the first volume of *Applied Artificial Intelligence*. To assess the journal's profile I cross-classified them by topic area, namely knowledge systems versus natural language, and also by category, namely (i) think-pieces (roughly equivalent to 'theoretical' but without the rigour) and (ii) annotated illustrations and evaluations (roughly equivalent to 'Look Ma, no hands' but without the derogatory connotation). Ten of the 20 were think-pieces about knowledge systems: something here, perhaps, of the more philosophical leaning sometimes attributed to the European mind. As well as its intended readership of researchers, I suspect *Applied Artificial Intelligence* will attract a following among the swelling ranks of interested non-specialists, the kibitzers of the game.

The next two publications, *Artificial Intelligence Review* and *The Knowledge Engineering Review*, exemplify the response in recent years to the kibitzer market, which has been unexpectedly voracious. The idea, new to scientific practice so far as I know, is to publish literature in journal style which is specialist in subject matter and yet addressed to the non-specialist. The maiden editorial

in *Artificial Intelligence Review* lists the main journals of the old kind, notably the *Artificial Intelligence Journal* and *Cognitive Science*. It then continues "These established publications . . . cater for the 'insiders' in the field. Public awareness and interest in AI however, has grown rapidly. There are now many 'outsiders' who would like to learn about AI. This is basically the rationale behind the launch of *Artificial Intelligence Review*". The statement of publication policy in *The Knowledge Engineering Review* makes no designation of target readership. But content and style leave little doubt that the primary alignment is the same.

My doubts about both of these journals stem from a lack of belief that the best way of conveying to outsiders the state of play on the inside is through the intermediary services of a special breed of commentators and debaters. Rather than listen to experienced motoring correspondents explaining in public discussion how the land lies between Aix and Ghent, I personally would settle for a scale map. If the outsider is serious, there is the option of subscribing to some abstracting service which covers the more significant of the latest technical communications. For example, through a collaboration between the International Management Institute, and my own laboratory, 'scale maps' (AI abstracts) have been prepared specially for a readership far outside the AI field.

With *Machine Learning* we turn from commentaries and maps to the matter itself — some would say the heart of the matter. The case for centrality is well expressed in Pat Langley's editorial introducing the first issue:

One of the major insights of both fields [artificial intelligence and cognitive psychology] has been that, except in the simplest domains, intelligent behaviour requires significant knowledge of those domains By re-focusing their efforts on learning, many researchers hope to discover more general principles of intelligence. In the case of psychology, such principles would lead to more encompassing theories of human behaviour that move beyond particular domains. In the case of applied AI, general learning methods might let one automate the construction of knowledge-intensive systems, saving man-years of effort for each application area.

The explicitly knowledge-centred definitions of AI exclude a variety of blind adaptive mechanisms to which a biologist might be happy to apply the term 'learning', including parameter-optimization, statistical pattern-recognition, neural nets and the like. A machine system which learned to *discuss* the dynamics of the bicycle would certainly qualify under the *Machine Learning* rubric. But what if it acquired solely the operational know-how of bicycle-riding? Empirical learning of this kind, analogous to skill-acquisition in human beings, figures in the journal's field of view only to the extent that the

system can display the heuristic models it has acquired in intelligible and transmissible symbolic forms. Rules induced from example data should approximate to what humans would call concept descriptions.

The rule-induction approach to empirical learning, pioneered by R.S. Michalski at Illinois and by J.R. Quinlan in Sydney, is well represented in *Machine Learning*. Beyond heuristic know-how, automated acquisition from data of causal and descriptive models can be made to lead from machine learning to machine discovery. It is pleasing that the great founder of this school, Herbert Simon, is a contributor of new work to the journal's first issue. Most gratifying of all is the evidence of progress in readying machine learning for its coming role in scientific investigation — for which it must itself become more scientific. In a guest editorial to a notable special issue of *Machine Learning* (Vol. 2, No. 4, April 1988), David Haussler remarks that "there is an art to designing efficient and effective learning algorithms. Too much of an art. Many researchers have argued that if the field is ever to fully mature, we must find a sound and adequate basis on which to build a science of machine learning".

That the building proceeds apace can in part be judged from papers in this landmark number of *Machine Learning*. On the experimental side, a surge of even more recent publication attests to the quickening tempo. I would prescribe at least a glance through the 50 or so communications to the *Fifth International Conference on Machine Learning* just published by Morgan Kaufmann. The 'helps for the mind' theme is evident, as in the communication by Cheeseman and colleagues of machine discovery from raw stellar spectral data of new categorizations of stars. These categorizations were new to the NASA astronomers and recognizably of clear physical significance.

In envisaging the future edifice of scientific knowledge, Francis Bacon knew that it could only be constructed at whatever pace might be permitted by advances in instrumentation. In modern times the term instrumentation is extending into the burgeoning world of scientific software. But Bacon also saw that ultimately *instrumentation of the intellectual process itself* would hold the key. His list, appended to his *Advancement of Learning* of the "deficiencies of knowledge . . . to be supplied by posterity" contains the item "The True Use of Induction in Philosophy" followed closely by "A New Engine; or helps for the mind corresponding to those of the hand". Bacon's motivation was practical. Four hundred years later we are seeing the first conversions of practicality into practicability. □

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Tapping into three dimensions

William C. Ripka

Journal of Computer-Aided Molecular Design. Editors-in-chief G.R. Marshall, J.G. Vinter and H.-D. Höltje. ESCOM, PO Box 214, 2300 AE Leiden, The Netherlands. 4/yr. Dfl 380, \$190.

THE phrase 'computer-aided drug design' is becoming one of the most abused in the English language. Although it is to be seen in the titles of several books and journal articles, one is often hard pressed to find any further mention of the design of any molecules beyond the words in the title. In fact, this is a legitimate and promising arena of research, which may provide the necessary link between the findings of molecular biology and conversion of this knowledge of macromolecules into the production of smaller, more manageable molecules.

Articles that have actually dealt squarely with the subject have been spread among an assortment of journals primarily serving other subjects (biology, physical chemistry, chemical synthesis, computer graphics, protein structure and so on). The object of the *Journal of Computer-Aided Molecular Design* is to rectify this unsatisfactory situation by bringing together reports of research directed towards all of the applications implied by its title. The editors state that the purpose of their journal is threefold — to be a forum for multidisciplinary communication, in recognition of the several areas that molecular design encompasses (computer science, chemistry, biology and structure-activity relationships); to provide a source of references to aid researchers in keeping abreast of developments; and (interestingly) to catalyse the use of electronic publishing by including an electronic version of the journal which "will enable the 'reader' to view the figures on his own terminal in three dimensions".

With respect to the first goal, the editors have been remarkably successful. The quality of the contributions has been consistently high in the first year of publication, and the papers have indeed dealt with a wide range of topics — theoretical and computational chemistry, computer graphics, drug design and molecular modelling, associated techniques in protein engineering and structure-activity relationships in general. One possible pitfall in attempting to cover such a broad scope is that the prime intention, namely, to deal with molecular design, is lost sight of. Yet the editors have managed to keep the emphasis of most of the contributions, whether theoretical or applied, firmly on

the design problem.

In addition to research contributions, a "Perspective" section is included in which appear excellent short summaries of important new topics; those by van Gunsteren *et al.* (Vol. 1, July 1987) on free-energy perturbation and by Donnelly and Kelder (Vol. 1, Oct. 1987) on distance geometry analysis of ligand-receptor site binding are especially good. The overall quality of production is excellent, with crisp colour photographs of computer graphics being included.

One of the most admirable of the journal's goals is to provide machine-readable results (ASCII files on IBM or Macintosh disks) from the various molecular modelling and enzyme-binding studies reported in the articles. The availab-

JOURNAL OF COMPUTER-AIDED MOLECULAR DESIGN

ility of coordinates from these investigations should allow access to the research in a much more usable form than the usual diagrams and two-dimensional views, and should encourage further refinements and studies of the models concerned. The availability of this machine-readable data from the first volume was announced in the April 1988 issue.

For both contributors and subscribers, *Journal of Computer-Aided Molecular Design* fills an important role. Further, by making the actual coordinates of the model studies available via ASCII files the journal is pioneering the communication techniques of the future. It should be considered essential reading for anyone interested in molecular modelling and molecular design. □

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Journal of what?

William H. Press

Journal of Scientific Computing. Editor Steven A. Orszag. Plenum. 4/yr. US \$135, elsewhere \$155.

LET US recall the old tale of 'nail soup', wherein an enterprising stranger astounds the rustic villagers by making a delicious soup from only boiling water and a nail — plus, of course, the various ingredients that they trade him for a chance to taste the miraculous pottage. The launch of a new scientific journal is something like that: publisher and editor seed the first issue with a few invited papers — not necessarily of the quality or breadth that they hope to attract — and then wait expectantly for an influx of tasty contributions.

Like the enterprising stranger, a founding editor tries to promise a lot, sometimes more than he can deliver: the *Journal of Scientific Computing*, wrote the editor, Steven A. Orszag, in its first issue, "is the forum for state-of-the-art developments in scientific computing and its applications" (italics his). That has simply not transpired. What we have instead is a modest, highly specialized journal that is firmly centred on Orszag's research interests, which I would take to be (i) computational fluid dynamics, (ii) especially incompressible flows, (iii) especially spectral (for example Chebyshev) methods. This is an interesting and active area; but by no stretch of the imagination can it be said to be the unique centre of 'scientific computing', whatever that is.

Some of the more interesting papers published, at least by the end of 1987, are

variations that range more widely from the theme, for example those on the implementation of some techniques on parallel computers and on efficient methods for some eigenvalue problems. The occasional paper on something as different from fluids as multimode waveguide analysis seems, however, to be the exception and not the rule.

Indeed, what is the core of scientific computing? Is it computational fluid dynamics? Is it finite difference approximation to partial differential equations? Is it image deconvolution, or three-dimensional visualization? Is it quantum chemistry, or geophysical fluid dynamics, or



lattice gauge theory, or Monte Carlo, or the collection of things represented in the well-established *Journal of Computational Physics*? I suspect that as regards defining scientific computing as a field in 1988, we are not yet beyond Justice Potter Stewart's definition of pornography in 1964 (*Jacobellis v. Ohio*): "... I know it when I see it".

The world does need a journal of scientific computing, but a broader consensus on what that field includes is surely a prerequisite. For now, specialized journals — even when as grandly named as this one — remain the rule. □

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Tools of the trade

Edwin Metcalfe & Stephen J. Haswell

Journal of Chemometrics. Editor-in-chief Bruce R. Kowalski. Wiley. 4/yr plus supplement. £125, \$225.

Chemometrics and Intelligent Laboratory Systems. Editor-in-chief D.L. Massart. Elsevier. 8/yr. Dfl 564, \$275.

CHEMOMETRICS is usually defined as the application of statistical, computational and mathematical methods to analytical chemistry, which in its broadest sense includes applications to areas as diverse as geochemistry and microbiology. The subject has undergone a period of tremendous growth and maturation over the past few years, so it is no accident that two journals devoted to it should appear recently and almost simultaneously.

Both *Journal of Chemometrics* and

Chemometrics and Intelligent Laboratory Systems provide wide coverage of the area through the publication of original papers and general information. Most of the articles in both journals are concerned with multivariate data analysis and reduction methods such as principal component, partial least squares and cluster analysis. Much of this work is aimed at extracting the essential information from multi-dimensional data sets for the purposes of classification, calibration or prediction. The alternative approaches of experimental design and optimization, in which the object is to reduce the experimentation required to yield the necessary information, are represented, although to a lesser extent. Other areas covered include the assessment and applications of expert systems and artificial intelligence, and signal-processing methods such as Kalman filtering and correlation chromatography.

The editorial approaches differ, in that

Journal of Chemometrics is more theoretically orientated; it is more formal in its presentation, and although the papers identify analytical problems their emphasis is more on the underlying theory. *Chemometrics and Intelligent Laboratory Systems*, on the other hand, contains a roughly equal mix of theoretical and applied papers; here the stress is upon the more practical aspects, as emphasized by the excellent tutorial section. This section addresses specific chemometric techniques, such as optimization of HPLC separation or soft modelling, and describes their use in a readable and comprehensible way. Both journals have book and software reviews and a diary section, but these are better presented and far more detailed in *Chemometrics and Intelligent Laboratory Systems*, which also includes a useful and sometimes provocative editorial slot, and meeting reports. In this way the journal appears to operate as an information forum for members of the Chemometrics Society, which acts as its sponsor.

Many of the original papers in both journals are of a high standard and could, with stylistic changes, be published in either one of them. Both have a reasonable rate of production, most articles and short communications being published within a year of receipt, and the illustrations and format are satisfactory.

These two journals are welcome in providing a forum for practising chemometricians, but it is both inevitable and necessary for the continued growth of the subject that many papers, particularly those which produce a faster or more reliable interpretation of real data, will continue to be published in other journals, to maintain and generate further dialogue with non-chemometricians. If chemometrics can be seen as essentially a tool kit, *Journal of Chemometrics* and *Chemometrics and Intelligent Laboratory Systems* are for reports of investigations into the advantages, developments and limitations of the various tools, while papers reporting the use of the tools as aids to wider investigations will continue to appear in analytical and more general journals.

Both of the journals reviewed here are reasonably priced and are essential reading for the practising chemometrician. The part-time chemometrician who is interested primarily in practical applications should, ideally, also consult both of them. But, if forced to choose, we would plump for *Chemometrics and Intelligent Laboratory Systems*, not just for its more applied nature but for the tutorials provided. □

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Compound interest

O. F. X. Donard

Applied Organometallic Chemistry. General editor P. J. Craig. Longman. 6/yr. UK £132, US \$264, elsewhere £139.

ORGANOMETALLIC compounds were a subject of interest as long ago as 1760 (*liqueur fumante de Cadet*). These days they are widely included in polymer systems as coupling agents, catalysts, cross-linking agents and biocides. The high toxicity of some of these compounds, and the possibility of natural alkylation of metals such as arsenic, mercury and tin, has resulted in intensive research on their fate and pathways in the environment — not least of the stimulants to this research were the disasters, such as that at Minamata, caused by pollution from organomercury compounds.

The editor and publisher of *Applied Organometallic Chemistry* have taken up a difficult and interesting challenge in attempting to reflect the wide variety of topics that their journal's title takes in. Subjects of papers published to date have included organometallic synthesis, industrial applications, toxicity effects on biota, environmental pathways and analytical chemistry. Most of the contributions

could have been published in a variety of other journals, but here they are offered to the reader in a single place.

The editor's aim is to produce papers "to the highest international standards". Judging from the first two volumes, these standards are being met, both in terms of scientific quality and level of the journal's production. The quality of the science is likely to be guaranteed by the distinguished editorial board, though it should be said that many of the members work in the environmental sciences, which may prefigure the future course of the journal. Delays for publication of contributions are very short at the present time, and a wide international selection of authors has already been on offer.

The journal publishes original research papers and reviews, and there is a place for short papers. Also included are book reviews, announcements of conferences and a list of papers due to appear in forthcoming issues. One welcome feature, though, which the journal lacks at

applied organometallic chemistry

present, would be a correspondence section to introduce some liveliness and encourage exchange between the contributors.

Applied Organometallic Chemistry promises well, though its future success will depend on maintaining the balance of papers on different topics. This journal will be a valuable tool for scientists working on any aspect of organometallic chemistry, but, because it is reasonably priced, it should also find a place in libraries covering the literature on trace metals in the environment. □

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Material gains

Robert W. Cahn

Materials Science Reports. Coordinating editors S.S. Lau and F.W. Saris. Elsevier. 8/yr. Dfl 307, \$149.75.

MATERIALS Science Reports, which began publication in September 1986, consists of issues 50–120 pages in length, each containing just one substantial review. All reviews are commissioned by the editors (one American, one Dutch); while suggestions for topics are solicited from the readership, uncommissioned contributions are not. The new journal thus sits in that shadowy middle ground between books and primary research journals, between publications that depend on contracts and those that depend on unsolicited submissions. Let us call them 'commrevs'.

There are already some commrevs in the materials field: the one closest in concept to *Materials Science Reports* is *CRC Critical Reviews in Solid State Sciences*. This serial has been published for several years, though its frequency of appearance seems to be somewhat less than that of *Materials Science Reports* and its reviews are rather shorter. *Progress in Materials Science* is another; at 39 years of age this has certainly stood the test of time, though its frequency of publication is gradually declining.

The editorial in the first number of the newcomer indicates that all categories of materials, all kinds of properties and their assessment, all forms of preparation are fair game for review. To judge from the early issues, the editors in fact intend that

their journal should have a somewhat applied orientation.

The splendid opening review by Buschow, on novel permanent-magnet materials, is evenly divided between engineering considerations and basic discussion of crystal structures and the quantum mechanics of spin–spin interactions. Tsuda's remarkable article on new polymers for use as radiation-sensitive resists for microlithography combines the methods of the theoretical chemist, who can interpret the spectral response of a molecule from first principles, with a practical presentation of the use of such resist materials: how much of the science is *a priori* and how much *a posteriori* is not quite clear, but that is characteristic of such essays into design of materials. Other articles cover, for instance, metal-organic vapour phase epitaxy of compound semiconductors and lattice sites of elements in forced (ion-implanted) solution in metals.

Overall, *Materials Science Reports* is rather more applied in flavour than the two other commrevs I've mentioned, though with solid scientific underpinning (indeed, the scientific level of all three journals is excellent). The quality of English, of printing and of illustrations is all one would expect from Elsevier. □

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Journal prices

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1989. This information is not complete in all cases. Readers interested in a particular journal should therefore check prices with the publisher before subscribing.

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On the search for solutions

Anthony Stone

Journal of Mathematical Chemistry. Editor Dennis H. Rouvray. *Baltzer, Wettsteinplatz 10, CH-4058 Basel, Switzerland.* 4/yr. SwFr 344.50, \$222 (institutional); SwFr 120, \$77.50 (personal).

MANY of the mathematical techniques of physics and chemistry can be applied to a wide variety of problems, and it sometimes happens that a development in one area turns out to have been anticipated somewhere else entirely. There is a case, therefore, for a journal in which such developments can be published. The *Journal of Mathematical Physics* has served such a purpose for many years, and it may be argued that because many basic techniques (such as group theory) are common to physics and chemistry, there is no need for a journal devoted specifically to the mathematical aspects of chemistry. But although the basic principles of group theory are common to high-energy physics and inorganic chemistry, the particular symmetry groups and applications are now so different that the fields have little in common. Moreover, modern chemistry depends more and more heavily on mathematics. In principle, then, the introduction of the *Journal of Mathematical Chemistry* is a sensible idea.

I was discouraged, though, to see that four of the eight papers in the first issue were on graph theory. This is a contentious subject, and here is not the place to

discuss it, even if I were qualified to do so. However, three of the papers concerned deal with the graph-theory version of simple Hückel theory, which played an important part in its time but which has for years been recognized as a grossly inadequate model of electronic structure. I was surprised to see it suggested by one of the authors that Hückel himself derived his model from graph theory, which I believe to be quite untrue. In fact, graph theory has yet to contribute to the body of fundamental ideas that constitute the chemist's intuition.

In fairness it should be said that graph theory is not so prominent in the other three issues that I have in front of me. Some of the topics will be regarded as fairly esoteric by the average chemist — knot theory of molecules and chirality polynomials, for instance — but there are also contributions concerned with statistical mechanics and thermodynamics, electronic structure, chemical equilibrium in multicomponent systems and the chemical kinetics of nerve synapses. The papers on knot theory and chirality polynomials are clear and well written, and are examples of the reviews which appear at the beginning of some issues. Such reviews serve a helpful function for chemists who are interested in finding out about mathematical developments that may be useful to them, and are a valuable feature of the journal. Nevertheless, I suspect that most chemistry library committees will decide that, at its not inconsiderable price, they can manage without it. □

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Mass movement

Robin Thain Aplin

Rapid Communications in Mass Spectrometry. Editor-in-chief John H. Beynon. *Heyden.* 12/yr. £103, \$165.

MASS spectrometry continues to develop apace and is finding wide application in many fields outside organic chemistry, in particular in the molecular sciences and environmental analysis. The advent of a journal with the specific aim of rapid publication of submissions was greeted with great enthusiasm at the American Society for Mass Spectrometry meeting in Denver in May 1987. For historical reasons, the publisher is most appropriate — in 1968, Heyden launched *Organic Mass Spectrometry*, the first specialist journal devoted to the subject.

The new journal's novel concept of 'sponsor referees', whose names appear after the title of the paper, has resulted in

a high scientific standard both of these papers (43 of them) and of the 25 normally refereed papers published in the first year. The contributions have covered a wide range of topics in all areas of mass spectrometry, and the aim of rapid publication has been more than met; most papers are published within five weeks of receipt, compared with six to twelve months for the other main mass spectrometry journals (*Organic Mass Spectrometry* and *The International Journal of Mass Spectrometry and Ion Physics*). Each issue also contains a diary of meetings, while a new product section and book reviews are included from time to time.

This is a modestly priced, high-quality production which gives excellent value in terms of its scope and the quality of its contents. It is a 'must' on the reading list of all those involved in mass spectrometry and its applications. □

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Going global

Thomas J. Crowley

Climate Dynamics. Executive editors W.L. Gates and H. Oeschger. *Springer-Verlag.* 4/yr. DM282, \$170.

IN THE past ten years climate studies have evolved from being dominated by meteorologists into a discipline that incorporates physical oceanography, geochemistry, cryosphere-biosphere interactions and palaeoclimatology. Progress has been greatly stimulated by realization of the significance of anthropogenic-induced changes, especially the greenhouse effect. There have also been substantial theoretical advances which have stemmed in large part from the availability of supercomputers. As the field has branched out and matured, it has devel-



oped an identity different from traditional 'climatology'; thus the name 'climate dynamics'. It is now at the stage where it is one of the key building blocks of the emerging 'global change' programme.

To some extent, climate research has outgrown a number of the journals and organizations whence it evolved. Some articles are certainly still appropriate for the long-established journals, but others, especially those of an interdisciplinary nature, need a new forum. *Climate Dynamics* aims to meet that need, putting considerable emphasis on interdisciplinary work and on the importance of theoretical advances.

In the hope of giving the journal a strong start, the editors have assembled a distinguished group of scientists for their editorial board. With five issues now published, it is safe to say that in general their goals are being realized. Most of the articles are of good-to-high quality, and the topics wide-ranging, although studies of the greenhouse effect and of palaeoclimates have predominated. A sampling of article content gives evidence to the broad scope — coupled ocean-atmosphere models, an ocean-carbon cycle model, past deep-water circulation changes, instabilities in climate models, sea-ice parameterizations, transient effects of CO₂ warming, ozone changes, analysis of historical data sets, and modelling of the African monsoon. To their credit, the editors have welcomed idealized mathematical models that often fall outside the limits of typical atmospheric science journals. Most of the

papers are 10–20 pages long, although there are a few longer ones. As yet no review articles, book reviews or editorials have appeared.

The journal is nicely produced — format is pleasing, illustrations are clear and the paper quality is very good. There is some room for improvement, however. Publication time for papers could be shortened from its present average of about a year, and the number of them in each issue could be increased from the present level of four or five. But my only serious complaint is the high cost for

private subscribers, especially those in the United States. Against that, contributors benefit from 50 free offprints, with reasonable charges for larger numbers.

It is clear that there is a need for a journal of this type, and that *Climate Dynamics* has the support of the most prominent members of the community. It promises to be an important addition to the list of climate and interdisciplinary research publications. □

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Period coverage

Rhodes W. Fairbridge

Journal of Quaternary Science. Editor J.J. Lowe. *Longman*. 3/yr. UK £79, US \$158, elsewhere £83.

A NEW journal dealing with Quaternary science — is not the area, like so many others, already abundantly supplied with outlets for our genius? Even in these days of overwhelming quantities of scientific papers, the answer is 'no'. With the pages of the high-profile, general journals of science closed to all except the most original, topical and closely worded contributions, there is still a tremendous need for space for the more specialized, the more regional and the more esoteric contributions.

Editors and reviewers must constantly remind themselves that the 'modest note' of today may contain that germ of an idea which blossoms a few decades later into the central axiom of a new theory. To my great delight I recently came across a fundamental paper on rainfall in the eighteenth century, in which the author recognized the 18.6-year lunar declination linkage (through atmospheric pressure). The publication date? 1832! Yet it is only during the past decade that this concept has been independently rediscovered. For editors, the moral of the story is clear: their duty is to sift wheat from chaff, but to reject a precious grain may verge on mortal sin. And if that grain is passed over by one publication, there ought to be plenty of other places for it to take root.

I for one welcome *Journal of Quaternary Science*. The Quaternary encompasses far more than just a geological period, although its scientific understanding rests squarely on the fundamental laws and axioms of geology. It demands astonishing interdisciplinary study, which explains the diversity and abundance of journals that accept papers relating to the subject. The newcomer has a place among them.

The journal is clearly the child of the

(British) Quaternary Research Association, although its intent is avowedly international; besides the British editorial board, it has an international advisory panel that includes Americans, Scandinavians and others, although none at all from the continental EEC area. So this is a rather anglophone club; let us hope the European integration of 1992 will also bring with it enriched intellectual co-operation.

Two numbers of *Journal of Quaternary*



Science were generated annually in 1986 and 1987, but three are promised for 1989. Style is orthodox and both editorial control and production are first class. Most of the papers are interesting and readable, and the illustrations are excellent and abundant. There are also some useful book reviews.

All this adds up to make a prominent journal that is likely to survive the critical test period (usually three years to make or break). Copies should be found in all libraries of geology, geography and botany, as well as in offices of environmental management and survey. The subscription rate is high — not surprisingly, given the production quality and the specialist audience — but a more adventurous (global) editorial thrust might expand the readership and, perhaps, bring down the price. Likewise, the inclusion of a calendar of forthcoming events and news of INQUA, and Quaternary IGBP activities could broaden the journal's appeal. □

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It's a small world

Lee Kump

Global Biogeochemical Cycles. Editor James J. McCarthy. *American Geophysical Union*. 4/yr. US \$105, elsewhere \$135 (institutional); US \$34, elsewhere \$64 (personal).

THE transport and transformation of chemical species during Earth surficial processes can be described by the concept of biogeochemical cycles. Their study has recently taken on a new and global significance because of the pressing need to understand the consequences of increasing human involvement in the Earth's system.

The stated purpose of *Global Biogeochemical Cycles* is to stimulate collaboration between marine, atmospheric, geological and biological scientists, and to improve our understanding of the linkages between Earth's physical climate and the biosphere. Indeed, Vol. 1 of the journal includes authors from marine science, geoscience, chemistry, environmental science, applied science, physics, agriculture, biology and meteorology departments, as well as from industry and government laboratories. Of the 26 papers published in 1987, almost half were written by co-authors from different disciplines.

According to the index of the first volume, the general topics covered (and the number of pages devoted to them, with some papers cross-listed) include atmospheric composition and structure (202), biological and chemical oceanography (158), general oceanography (100), meteorology and atmospheric dynamics (72), physical oceanography (24), marine geology and geophysics (18), hydrology (18) and public issues (14). Terrestrial systems don't receive separate billing, but 20 per cent of the articles describe land-atmosphere interactions.

Global Biogeochemical Cycles apparently has had some problems getting off the ground. A third of the papers display acceptance dates that postdate the nominal publication date by a month or more. The journal's survival will depend crucially on a rapid improvement in the promptness of publication and distribution.

The quality of the papers that have been published is high, and the camera-ready copy is clear and well reproduced. The journal meets an urgent need, in that it is a place to publish and to read interdisciplinary papers addressing global biogeochemical problems. The scientific community can either help it to flourish, by submitting more high-quality manuscripts, or condemn it, by publishing in conventional, disciplinary journals. □

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A couple of cross-pollinators

Jerry A. Coyne

Evolutionary Ecology. Editor-in-chief Michael L. Rosenzweig. Chapman & Hall. 4/yr. UK £60, US \$110, elsewhere £67.50 (institutional); UK £25, US \$37.50, elsewhere £30 (personal).

Trends in Ecology & Evolution. Editor Andrew M. Sugden. Elsevier. 12/yr. UK and elsewhere £144, US \$265 (institutional); UK £29, US \$53, elsewhere £37 (personal).

ECOLOGISTS and evolutionary biologists would seem to be natural partners, but do not often have much to do with each other because few meetings and journals appeal to mutual interests. The problem has been lessened by the introduction of these publications. Despite their similarity of title and price per page, they are very different: *Evolutionary Ecology* is a vehicle for primary research, while *Trends in Ecology & Evolution* is a secondary



source that summarizes and calls attention to research published elsewhere.

Evolutionary Ecology is devoted to "conceptually oriented" papers, that is, proposals of new theories or descriptions of data designed to evaluate theories. Each 100-page issue includes about seven papers. The journal does not print review papers or book reviews, but does publish special symposia (for instance, a recent issue devoted to habitat selection). Other papers deal with the evolution of sex ratio, optimization, competition, and sexual selection — topics familiar to readers of *The American Naturalist*. Indeed, the content of these two journals is almost identical, the editors of *Evolutionary Ecology* having chosen the route of competition instead of niche shift.

How does the new journal fare in the competition for good papers? The quality is more variable than it should be, with the contributions ranging from the truly excellent and provocative (most of these being data-orientated) to those flawed by substantial errors in reasoning. In addition, there are too many theoretical studies that have no obvious relationship to real organisms. This makes the average quality somewhat lower than that of *The American Naturalist*, but things may change as *Evolutionary Ecology* ages.

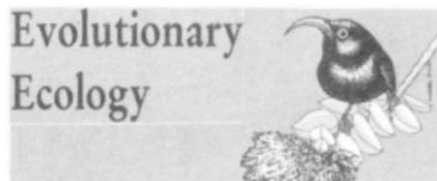
The source of the journal's problem is twofold. First, the editor's desire for rapid publication, although admirable, has produced a policy of critical review by only a single associate editor. Yet multiple refereeing is the surest route to improving manuscripts, and might have prevented

some unfortunate errors in the first few issues. In addition, the journal's statement of purpose implies a much higher respect for theory than for data: "Facts may endure, but what does raw fact do to advance science? José Clemente Orozco could well have been summarizing the philosophy of this journal when he wrote: 'Errors and exaggerations do not matter. What matters is boldness in thinking' " (Vol. 1, p.3). Such a cavalier attitude towards the facts is unbecoming of a journal devoted to understanding organisms in the wild.

It is hard to get new publications off the ground, and the editors of *Evolutionary Ecology* can be excused for some early lapses in quality. Nevertheless, if it is to enter the first rank of journals it must either harmonize rapidly with quality, or sacrifice speed by encouraging multiple review of submissions.

Trends in Ecology & Evolution, on the other hand, is uniformly excellent, having established itself in only two years as a 'must read' journal. It is designed to keep evolutionary biologists and ecologists up to date in both fields despite the explosion of publications relevant to them. Each 30-

40-page issue includes brief research notes about single papers (similar to *Nature's* News and Views section), three or four longer review papers on more general topics, book reviews, and occasional letters and commentaries on previous papers. Some issues are devoted to single areas (for example, evolution on the Hawaiian Islands). Regular topics covered include not only those that are trendy (sexual selection, genetics and conservation, primate infanticide), but also — to the editor's credit — fascinating areas that are not widely appreciated (gut



fungi, the effects of air pollution on plants, the evolution of bird song). The quality of the articles is very high, especially considering that this is not a journal of primary research, and the review articles not only summarize the literature but evaluate it critically. Special praise should be given for the graphics, which are the best of any journal in the field.

There have been a few weak articles, to be sure, but I find myself reading each issue in its entirety, something that I rarely do with journals in my own area. *Trends in Ecology & Evolution* deserves to be looked at (and subscribed to) by every population biologist. □

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Eclectic ecology

J.S. Jones

Functional Ecology. Editors P. Calow and J. Grace. Blackwell Scientific. 6/yr. UK £92, US \$189, elsewhere £110.

Functional Ecology is a slightly odd title — what other kind of ecology is there? Still, at least there is no mention of the word 'environmental', the kiss of death for any serious scientific endeavour.

Judging from its natal year, the journal is succeeding in its expressed aim of covering the physiological, biophysical and evolutionary aspects of ecology. Papers published to date deal with topics as diverse as wilting in tropical trees, the ranging behaviour of tortoises, and why a cladoceran is not a bang-bang strategist when it might be expected so to be.

Functional Ecology is unusual in that, as well as standard research papers, it contains a regular series of essay reviews (the first of which is an attempt by one of the editors to define exactly what functional

ecology is) and a 'forum section' (first occupied by two eloquent critics of the editor's definition of his subject). In addition there is a series of technical reports, which tell us how to measure the fat on a hungry gull, the speed of a sprinting lizard, and the effectiveness of plants in dealing with acid rain. The journal is elegantly produced, and is a worthy newcomer to the British Ecological Society's existing stable of three successful and well-established ecological journals.

The ecological and evolutionary journal ecosystem is becoming crowded, with at least ten competing publications in the field. Each is affected to a greater or lesser extent by the topics exploited by the new arrival. As is the case with all invaders into an established community, only time can tell whether the future will bring the evolution of niche displacement to reduce joint dependence on a shared resource, or extinction; for in ecology, as in life, nothing succeeds like succession. □

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Reviews on IMMUNOASSAY TECHNOLOGY

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Policing the planet

Kenneth Mellanby

Conservation Biology. Editor David Ehrenfeld. Blackwell Scientific. 4/yr. US \$90, elsewhere \$115 (institutional); US \$60, elsewhere \$85 (personal).

European Environment Review. Editor Cynthia Whitehead. Graham & Trotman. 4/yr. £92, \$165 (institutional); £32, \$56 (personal).

IN 1959 the British Nature Conservancy decided that a new research station was needed to work on problems related to scientific methods of wildlife conservation. The following year I was appointed as director of Monks Wood Experimental Station with the task of making that decision a reality. Soon we had nearly a hundred ecologists at work. Substantial scientific papers began to appear, and they were mostly published in the journals of the British Ecological Society. At that time there seemed little difficulty in finding outlets for the publication of our work.

Much more research on similar lines was starting in universities and research stations throughout the world, however, and new journals to accommodate its results were evidently required. *Biological Conservation* was started in 1968 by Elsevier Applied Science, with Nicholas Polunin as its editor. This journal's purpose was to "Disseminate original papers dealing with the preservation of wildlife and the conservation or wise use of biological and natural resources". It clearly met a need, for starting with four issues and 320 pages a year, by 1987 it had an annual output of 16 issues and some 1,400 pages.

Professor Polunin ceased to edit *Biological Conservation* in 1973, and started a further journal, *Environmental Conservation*, published by Elsevier Sequoia. This had a slightly different purpose, namely, "to maintain global viability through exposing and countering environmental deterioration resulting from human population-pressure and unwise technology". However, although it did contain papers dealing with damage to the global environment, and short notes reporting specific incidents, for the most part its longer articles were very similar to those continuing to appear in *Biological Conservation*.

In 1985 the Society for Conservation Biology was established in the United States and decided that it must publish its own journal, hence the appearance of *Conservation Biology*. An article by the society's president, M.E. Soulé, in the first issue, states that its purpose is to try to ensure that "the worst biological disaster in the last 65 million years can be averted". This would suggest that the

journal is more like *Environmental Conservation* than *Biological Conservation*; however, when describing the sort of paper the journal will welcome, Dr Soulé speaks of "Modeling and analysis of population, community, ecosystem and planetary processes" and also "basic field work, including inventories and systematics".

An examination of the first five issues of *Conservation Biology* shows that its pages are mainly devoted to solid ecological papers of up to 8,000 words in length. Although 18 of the 23 members of the editorial board are based in the United States, this is a genuinely international journal, with an apparently increasing number of contributions from India, Africa, Indonesia and other parts of the world. Notwithstanding Dr Soulé's comments about world disaster, the papers are almost all very similar to those appearing in *Biological Conservation*, except that they tend to have a rather higher theoretical or 'academic' content.

There seems no doubt that there was a place in the literature waiting for *Conservation Biology*. With the support of its parent society it is likely to have a viable market even if its other sales are small, but many of its papers are in fact likely to be required reading for ecologists throughout the world. Its price to members of the society is modest, and to libraries and institutions it is what would be expected today for a scientific publication of this nature which is excellently printed and presented.

The first journal considered here is aimed — successfully so — at an entirely scientific audience. The other, *European Environment Review*, has as its subtitle *The Journal of European Community and International Environmental Policy and Law*. It claims to be "an international, multidisciplinary journal which aims to provide a forum for the presentation of information and views on all aspects of environmental protection". It is published in English, French and German.

The journal does contain articles with a scientific basis — on radiation from the Chernobyl accident, on toxicity problems from heavy metals and other chemicals, on water pollution and other environmental dangers — but these are aimed at non-scientists and contain little information not already widely disseminated. The value of this publication, then, will be to administrators and international lawyers, and if it improves their scientific understanding and their knowledge of environmental problems, it is to be welcomed as making the work of scientists involved in these fields less difficult. □

Kenneth Mellanby, 38 Warkworth Street, Cambridge CB1 1EG, UK, was Director of Monks Wood Experimental Station from 1960 to 1974, and founder-editor of the journal *Environmental Pollution*.

Beyond the parish

T.C. Champion

Journal of World Prehistory. Co-editors Fred Wendorf and Angela E. Close. Plenum. 4/yr. US \$95, elsewhere \$110.

THE GROWTH in archaeological research throughout the world shows no sign of slowing down. One of the consequences of this information explosion has been something of a bibliographical nightmare.

Most research is still designed and carried out in a local context, and the results usually appear in regional or national journals, or monograph series. The multiplicity of languages used means that even the most gifted linguists among us cannot follow the literature, even assuming that we know what we are looking for and that the library can provide it. There are few truly international journals, and few regions where an effective abstracting service exists. This is particularly ironic at a time when archaeologists are increasingly interested in general patterns of social organization and general processes of change, rather than merely local sequences of culture history. The archaeologist working on general topics is therefore at a disadvantage, and it is all too easy to overlook relevant work through simply never knowing of its existence.

So there is a desperate requirement for better information services in archaeology, one of them being the need for a supply of reliable review articles. Until recently this service was to a large extent

provided by Academic Press's annual series *Advances in World Archaeology*, which, I understand, was discontinued after the appearance of Vol. 5. Now we have a new journal under the same editorship, but from a different publisher. *Journal of World Prehistory* very much retains the same style and contents as the book series, which makes it rather unusual for a journal. It includes nothing but review articles, two per issue in the first volume, thus giving authors 50 pages or more for detailed and critical discussion of recent research, and enabling them to include good bibliographies.

The journal covers prehistoric archaeology only, and within that the main interest seems to lie in the earlier periods and less complex societies. Geographical coverage is extensive. The first volume included papers on Europe, South America, northern Asia and island south-east Asia, as well as one on the techniques and potential of radiocarbon accelerator dating; they are generally well illustrated, though occasionally reproduction from original sources has given indifferent results, and the retention of untranslated captions in some figures looks rather odd.

There is no doubt that, for the good of the subject, such an outlet for reviews is needed, and the quality of the first volume of *Journal of World Prehistory* augurs well for its future. It would be a sad day if archaeologists were too obsessed with their own parochial interests to take advantage of a venture such as this. □

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Test-tube toxins

T.A. Connors

Toxicology in Vitro. Editors I.F.H. Purchase and G.C. Hard. Pergamon. 4/yr. £70, \$110 (institutional); individual rates also available.

Comments on Toxicology. Editors A. Wallace Hayes and William O. Berndt. Gordon & Breach. 6 issues per volume (see below). \$268 (corporate); \$190 (library); \$80 (personal).

TOXICOLOGY covers a broad area, involving not only research scientists of different disciplines, but industrialists concerned with safety evaluation and government bodies responsible for the regulation of the use of chemicals. Subsections of the subject are becoming more clearly defined, and have been marked by the appearance over the past few years of journals such as *Human Toxicology*, *Reproductive Toxicology* and the recently announced *Chemical Research in Toxicology*, a publication of the American

Chemical Society.

Scientists working on basic mechanisms of toxicity often also deal with the screening of chemicals for their biological properties and with safety evaluation of materials before they reach the market. Because *in vitro* methods are quick and cheap, and because they overcome the ethical problems of using animals, research in this area is burgeoning. *Toxicology in Vitro* is intended to cater for all those so involved. Its main intention is to publish original research papers on the use of *in vitro* techniques for determining the toxic effects of chemicals (safety evaluation) and elucidating their mechanisms of action (basic research).

The journal does not over-emphasize the development of alternatives to the use of animals, but a statement in the editorial of the first number makes it clear that one of its principal aims is to stimulate an improvement in the hazard assessment of chemicals using *in vitro* procedures. There is, however, the recognition that *in vitro* methods for the most part remain complementary to *in vivo* systems and may often be used for pre-screening prior to

toxicity testing in whole animals.

No new journal can be expected to publish high-quality work only, and the variation in scientific standard in the first two volumes is much what one would expect. The papers range from considerations of simple *in vitro* models for quantifying the properties of, for example, skin irritants, to more basic studies on mechanisms of action. As long as this balance is maintained then the journal should flourish. The stress will be on the publication of research papers, but brief communications will also be considered, and it is pro-



posed to include concise interpretative reviews from time to time. Publication speed is reasonable and the quality of printing, reproduction of figures and so on is similar to that of other Pergamon scientific journals — that is, adequate but not outstanding.

It is a much more difficult task to predict the future of *Comments on Toxicology*, because the journal is not really well known at present, amongst British toxicologists at least. Its aims are to present topical issues in a manner designed to stimulate discussion and critical examination. Because of the complexity of toxicology, there is certainly a need for a forum of this sort, and the journal has indeed published some interesting material — for instance the five papers in Vol. II, No. 2, devoted to a discussion of the importance of blind-slide reading in carcinogenicity and chronic toxicity studies. The general idea is that each chosen subject should be introduced by a guest editor who will set the stage for the theme under consideration. One criticism, however, is that this plan is not working too well at the moment; some numbers have not had guest editors, while the introductions to others have been short and unincisive.

I hope *Comments on Toxicology* succeeds because the idea behind it is a good one. But those libraries with diminishing budgets might feel it too much of a luxury and restrict their purchases to journals containing reports of original research. □

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• Harwood Academic Publishers and Gordon and Breach Science Publishers produce journals under the 'flow system'. Once sufficient papers are accepted to complete an issue, that issue is closed and quickly published. According to the publishers, this avoids the usual delays created by rigid schedules, but means that the number of issues per year cannot be accurately predicted.

The Pollyanna report

F.J. Bollum

Soviet Progress in Biochemistry. Soviet editor-in chief V.K. Lishko. Allerton, New York. 6/yr. \$395.

THE scope of this translation series is less than would be expected from the title, because it includes only selected articles from the *Ukrainian Biochemical Journal* rather than providing a comprehensive view of biochemistry in the Soviet Union*. As such it is an adjunct to the translation of *Biokhemika*, with which it shares some technical problems. Thus my most serious criticism is of the American publishers for producing this journal of substandard translation by substandard printing methods. Photo-offset printing of typed manuscripts and poor scaling and reproduction of figures makes for hard, sometimes impossible, reading. And the quality of translation requires additional concentration. One expects much better, given the state of computer-assisted word-processing and translation.

The papers translated include original

*From 1989, *Soviet Progress in Biochemistry* will be retitled *Ukrainian Biochemistry*.

work, brief communications and reviews of new methodology. Although these are said to be 'selected articles', there is no indication of the selection process, nor that anything less than the complete journal has been translated in the four issues I reviewed. The issues were published in 1987, so the experimental work reported in them was probably completed in 1985–1986. This double publication lag is expected in translation, but does put some bias on the reader's view of the currency of the information.

Forgetting for the moment all of these technical problems, what is going on in Ukrainian biochemistry? By Western European and American standards — not too much. One review article describes the virtues of immunoblot technology. With the extended uses of solid-phase substrates current in Western biochemistry (no pun intended), this article provides no new insight. The original papers often have an applied tone, being "The effect of [compound A] on the metabolism of [substance B]". One interesting example providing little comfort to space travellers and embassy employees is

"The Effect of Alternating Magnetic Fields of Industrial Frequency on Rat Liver Lipid Composition". Some of the interpretations are more fanciful than those allowed by refereed journals in the West. In many articles important experimental details, as well as the full address of the authors, are missing. So what you read, with difficulty, is all you get.

Soviet Progress in Biochemistry is not going to let Western biochemists know what Russian biochemists can contribute to our science. As progress in Western biochemistry is the direct result of sharing of information, reagents and trade, so perhaps *glasnost* and *perestroika* might result in some reversal of provincialism. Then we can relegate reading *Progress in Soviet Biochemistry* (in English) and *Western Trends in Biochemistry* (in Russian, if it exists) to the appropriate bureaucrats in Moscow, Washington and London, and get on with some real progress in Soviet biochemistry. □

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It's only natural

Leslie Crombie

Phytotherapy Research. Editor-in-chief Fred J. Evans. Heyden. 6/yr. £118, \$190.

MANKIND has been indulging in phytotherapy research for a long, long time, and the products of this research — traditional remedies — can be brews of a dozen or so useless plants along with one which contains an active and effective compound. Sorting out what, if anything, is effective in the remedy can be slow and difficult, but the occasional emergence of a compound with impressive pharmacological activity makes it all worthwhile.

In the editor's words, *Phytotherapy Research* is intended to fill a "need for a specialized journal to bring together the wealth of biological, therapeutic and analytical information on pure natural products, plant extracts and their pharmaceutical formulations". The journal publishes original research papers alongside rapid communications, reviews and book reviews. At the end of each issue there are two sections, a patent alert and a current literature bibliography. Given the widely scattered natural products literature relevant to this area, these sections are of great value for keeping up to date.

The journal will particularly appeal to pharmaceutical scientists. Some original papers report the screening of crude plant extracts, containing many chemical species, using a particular screen against certain disease conditions, thereby pro-

viding leads for later phytochemical investigation and for the isolation and structural analysis of active chemical components. Others examine the biological activities of pure chemical compounds isolated from plants, sometimes chemically modified further. The chemical interest, however, apart from illustrative formulae, is rather limited in the four issues I looked at.

The journal seems reasonably priced and the general presentation of articles is modern and attractive. Publication appears to be quite rapid, inasmuch as this can be judged reliably for a new journal. There are of course other unknown quantities. A range of good manuscripts can usually be organized for the first few issues, but only time will tell whether the quality and flow can be sustained and developed. The level and extent of refereeing is not made clear, although this is of great importance for a new journal trying to establish a good reputation.

Nonetheless, *Phytotherapy Research* undoubtedly fills a need. It will be especially attractive as a forum for scientists from less-developed countries where phytotherapy is widely practised. Collaborations between scientists in developed and less-developed areas of the world give rise to especially valuable studies in this area of research, and some of the papers in the issues I examined bear that out. □

Leslie Crombie is Sir Jesse Boot Professor in the Department of Organic Chemistry, University of Nottingham, Nottingham NG7 2RD, UK, and immediate Past Chairman of the Phytochemical Society of Europe.

Metastatic Spread of Cancer

G.V. Sherbet

This book outlines a new approach to the analysis of the mechanisms of metastasis by comparing the glioma, an intracranial tumour which ordinarily does not metastasize, to other metastasizing tumour models. This has produced a coherent picture of the operation of oncogenes and growth factors in the initiation, development and progression of neoplasms. This is an essential book for those researching metastasis and will be of interest to all research oncologists.

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From France and around the world

B.L. Ginsborg

Fundamental & Clinical Pharmacology. Editors-in-chief Salomon Z. Langer, Philippe Meyer and Jean-François Giudicelli. *Elsevier*. 6/yr. France Fr 885, elsewhere Fr 995.

ISI Atlas of Science: Pharmacology. Publisher and editor Alexander M. Grimwade. *ISI*. 4/yr plus annual compilation. \$125 (institutional); \$65 (personal).

EVERYTHING between the covers of *Fundamental & Clinical Pharmacology* (subtitled *The International Publication of the French Association des Pharmacologistes*), including the abstracts of papers presented at the association's meetings, is in English. Presumably French pharmacologists have decided that this was necessary to enlarge the pool of potential readers and contributors.

The four numbers sent to me for review contained 23 regular articles, varying in length between 5 and 16 pages, and two interesting reviews, one by Thesleff on recent work on large "miniature end-plate potentials" and the other by Schwartz on calcium channel drugs. About half of the contributions originated in French institutions; others came from Italy, Yugoslavia, the United States, Britain, Israel and Canada. I did not spot any paper for which I would predict an outstanding citation score, but the journal is well produced, has a strong editorial board and stands a good chance of being attractive to authors of short papers of high quality. All that the publisher and editors can now do is to hope for the appropriate response from volunteers.

That is not at all the case for the *ISI Atlas of Science: Pharmacology*, which consists entirely of commissioned articles. At first sight, this journal seems like the answer to a prayer. Each number contains about 20 short surveys of fashionable topics, selected, according to a prefatory note, by "sophisticated techniques" from "areas where research activity is most highly concentrated". The editorial advisory board is distinguished and the authors have been chosen for their expertise. In the compass of about four pages, each survey contains, amongst other components, a summary, a historical section and a set of references. It seems too good to be true; just four pages, or even fewer, for enlightenment.

The publishers assert that the *Atlas* will be "a 'must' for researchers" and "invaluable to graduate students", not to mention "educators and lecturers". Some, however, may be reminded of that series of little books that bear on their front cover

"Know your jargon and hold your own in any company — Instant Erudition". The title of the series? *The Bluffer's Guides*.

The first four issues provide cause for both enthusiasm and misgivings. On the negative side many of the articles are cramped, conclusions are stated without a hint of evidence and cartoons replace arguments. What is wrong is probably as much the fault of editorial policy as of the authors. Short of space as they are, the contributors are nevertheless expected to include frivolities such as a list of "Key players". In the words of one evidently reluctant author: "The identification of key players is very difficult and undoubtedly subjective. . . . Time, death, and obituaries will ultimately make the appropriate judgements". Space is also frequently wasted on excessively long lists of references, which furthermore often include books and symposium volumes, types of literature notoriously difficult to come by.

Another feature which seems to be inappropriate is the glossary. It is true that it allows compression, such as "In addition to FIAC, various other 2'-fluorinated arabinosylpyrimidine analogs, i.e. FMAU, FIAU, FEAU and FBVAU, have been found effective as potent and selective inhibitors of HSV and VZV replication". But is that so very desirable?

As well as what might be termed defects in editorial tactics, there are also strategic weaknesses. For example, three separate articles on voltage-operated calcium channels (which the subject is sufficiently complicated to deserve) rather than reinforcing each other, overlap irritatingly and sometimes confusingly. The individual articles are good but with a little editorial effort the ensemble could have been greatly improved.

On the positive side is the diversity of the material that is covered: the first volume includes articles on bacterial resistance, immunology, toxicity and psychopharmacology to name but a small selection of topics. And above all, there are some splendid contributions. One example, from the first issue, is "Behavioral Effects of Peripherally Administered Cholecystokinin" by J.E. Morley. This seems to me to be a model of what can be done. The author constantly keeps the reader aware of the experiments which underpin his conclusions, explains unfamiliar terms as he goes along and points to the broader implications of what might at first seem to be a rather narrow topic.

This is alpha double-plus stuff. In spite of its present faults, the pharmacological *Atlas* is a worthwhile venture, and potentially a very useful addition to the literature. □

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Periodical profusion

John B. Hall

Trees: Structure and Function. Managing editor H. Ziegler. *Springer-Verlag*. 4/yr. DM 248.

New Forests: Biology, Biotechnology, and Management of Afforestation and Reforestation. Editor-in-chief Mary L. Duryea. *Kluwer*. 4/yr. Dfl 208, \$101 (institutional); Dfl 110, \$55 (personal).

Tree Physiology. Editor Rozanne Poulson. *Heron Publishing*, Box 5579, Station B, Victoria, British Columbia V8R 6S4. 4/yr. \$132 (institutional); \$66 (personal).

Scandinavian Journal of Forest Research. Editor-in-chief Sven-Ulrich Skarp. *Amqvist and Wiksell*, PO Box 638, S-101 28 Stockholm. 4/yr. Skr 535, \$88 (institutional); Skr 600, \$98 (personal).

FORESTRY is a small and traditional profession. New journals dealing with the subject stand alongside publications with 50- or even 100-year pedigrees, almost all of them institutional or society organs. Forestry was largely unaffected by the proliferation of biological periodicals in the 1960s and 1970s, and has, in some respects, kept its tradition at the expense of topicality. The new journals considered here are signs that change is at least gaining momentum, and reflect the publishing fraternity's conviction that new



outlets are needed to cater for interests in trees and forestry extending well beyond professional ranks.

Trees: Structure and Function and *New Forests* emphasize the biological aspects of forestry. *Tree Physiology* expresses the same intention without recourse to the term 'forestry', except to point out that it is covered by *Forestry Abstracts*. Only the *Scandinavian Journal of Forest Research* pursues a conventional path in having institutional affiliations (the Nordic Forest Research Cooperation Committee and the Royal Swedish Academy of Agriculture and Forestry) and specifically stating that its field is basic forestry sciences.

In technical coverage, *Trees*, *New Forests* and *Tree Physiology* overlap both with each other and with established journals, but there are distinctions between them. *New Forests* stands apart in its adherence to routine forestry work and its stress on research in the field, which bring it close in aim to the well-established

Forest Ecology and Management. *Trees* and *Tree Physiology* are similar in content, but in these early issues *Trees* has mainly included contributions with an emphasis on field work with conifers, whereas *Tree Physiology* has attracted more papers dealing with broad-leaved species in controlled environments.

Geographical coverage in all four journals falls well short of the global, even though all of them imply that they have an international audience in mind (the *Scandinavian Journal* does, however, specify that contributions should be "of relevance to Nordic conditions"). The lack of contributions on work in the Third World is especially striking. In *Trees* and *Tree Physiology* (with one and two papers dealing with the Third World out of 43 and 30, respectively) a bias towards north-temperate research is evident but perhaps is to be expected. For *New Forests*, the



preponderance of North American work raises doubts over its ability to attract subscribers in tropical countries — only a single paper, out of 24, has a Third World emphasis.

Potential contributors can, in every case, expect competent editorial service. The published versions of papers are well-organized, and readable and direct in style. Illustrations and figures are clear, except in a few cases where reduction has obscured the distinction between symbols — a weakness not restricted to new journals. All are currently quarterlies and, as far as can be judged, submissions appear in print 6–12 months after receipt. Unless articles exceed eight pages (and then only for the *Scandinavian Journal*) there are no page charges. There is provision for review papers in all four, and for research notes in the *Scandinavian Journal*, *New Forests* and *Trees*. Book reviews featured in the issues of *Tree Physiology* and *New Forests* I looked at, and the latter also includes an innovative "Application" section. There must be innumerable journals where such a slot would be appropriate.

None of these journals addresses a new

Journal prices

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1989. This information is not complete in all cases. Readers interested in a particular journal should therefore check prices with the publisher before subscribing.

New Forests



field or bridges a gap. Forestry, however, has long been short of genuinely international outlets that demand the rigour and formality of research acknowledged in other disciplines. If these newcomers encourage innovation, stimulate interdisciplinary work towards the more efficient use of tree resources and raise the profile of forest research they will justify their existence. But I suspect that for all of them except the *Scandinavian Journal*, which is presumably protected by its affiliations, such an outcome will not depend on whether publishable material is forthcoming but on whether they can attract a viable number of subscribers. Forestry and its associated professions and scientific disciplines make only a small market, and journals with no institutional support need a world-wide circulation. From what

I have seen of *New Forests*, *Trees* and *Tree Physiology* I would like them to survive, but if they are to do so I believe they will have to appeal to contributors and readers beyond Australia, Europe and North America — after all, it is in the low latitudes that forestry must progress fastest to counter the effects of changes in land use, and where interest in trees is accelerating most sharply.

In that context, price becomes an issue. The system of subsidizing individual subscribers through higher rates for libraries has no relevance in countries where individuals have no foreign exchange and libraries have minimal resources. The publishers of these journals should note the precedents for offering reduced subscription rates for poorer countries. How many Third World libraries can pay \$37 for a 62-page issue of *Trees*, or, for about 90 pages of *New Forests*, seemingly the bargain of the three, even \$24 per issue? □

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Fungi for all

Alan D.M. Rayner

The Mycologist. Senior editor D.N. Pegler. Cambridge University Press. 4/yr. £8, \$16.

FUNGI probably rival flowering plants in their species diversity, and outweigh the animal kingdom. Whilst wielding great destructive power as agents of disease and decay, they drive the global carbon cycle, sustain our forests and grasslands via mycorrhizal associations, and clothe, as lichens, what would otherwise be bare parts of the planet. Their developmentally versatile body forms provide immense scope for industrial exploitation as well as experimentally accessible systems for studying fundamental biological issues. Yet most people's appreciation of fungi stops at mushrooms, mouldy food and fairy tales.

Challenged by such ignorance, mycologists need to overcome some deeply rooted prejudices. On the one hand, the variety, edibility and toxicity of fungal fruit bodies has always been a source of fascination which can be relied on to deliver new recruits to the cause of mycology. But if that fascination becomes an obsession, the cause is lost.

On the other hand, mycologists working on disease control, taxonomy or some industrial process often find it difficult to communicate the wider interest of what they are doing. Because of the vicious cycle of neglect, their task is made harder

by the need to use 'technical' terms: plant scientists can assume their audience knows what leaves, roots and stems are; mycologists always have to explain what hyphae and mycelium are.

So there are two common images of the mycologist — one of the eccentric amateur, the other of the remote professional working on esoteric problems. Both are damaging. By promising to present a more truthful picture and at the same time convey some of the real excitement of studying fungi, *The Mycologist* represents an imaginative and timely venture of the British Mycological Society. It appeals to all ages and educational levels, containing features on identification, illustration and consumption of fungi alongside book reviews, news items on mycological science and accessible reviews of important topics in fungal biology (recent examples being fungal biotechnology and ascomycete classification). Also published is news about the society's meetings and members.

A combination of journal, magazine and society newsletter, *The Mycologist* is inexpensive, yet well illustrated, with many high-quality colour photographs, and it deserves widespread circulation — perhaps especially in schools and colleges of education. Currently it is a little too parochial and the subject selection is too random for my liking, but on its showing so far it could well evolve to play a valuable role in the vitalization of mycology. □

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Are oncogenes in a class of their own?

Gordon Peters

Oncogene Research. Editors-in-chief Claudio Basilico and Hidesaburo Hanafusa. Harwood. 4 issues per volume. UK £138, North America \$224, elsewhere £170 (corporate); UK £84, North America \$136, elsewhere £106 (library); UK £42, North America \$70, elsewhere £54 (personal).

Oncogene. Editors Graham Currie, John Jenkins and Prem Reddy. Macmillan, London. 12/yr. UK £190, elsewhere £210.

AT THE risk of sounding like Peter Duesberg, what exactly is an oncogene, and why are oncogenes so special as to warrant two new and exclusive journals? The believers would claim that oncogenes are special because they may have important roles in the development of cancer, or because studying them at least promises to add to our understanding of the processes involved. Given the rate at which information on these genes has been accruing, there has been a clear case for a forum in which the main advances might be brought rapidly and reputably to the



attention of the specialists, particularly as the 'scientific tabloids' can be rather fickle in what they regard as topical.

There is, however, the counter argument that to elevate oncogenes into a class of their own is misguided. Most of the genes that receive this accolade are really not that special, at least not in the way they are organized or expressed; and what a mixed bunch they are too — growth factors, receptors, protein kinases, DNA-binding proteins, transcription factors, and genes whose functions remain mysterious — all catapulted to fame and fortune by tumour viruses and cancer funding. In point of fact, many members of this apparent élite have more in common with ordinary, run-of-the-mill genes than they do with one another, and to issue them with a separate passport disguises this reality. Take the case of cellular oncogenes that belong to large families — should there be a family passport, or should members who cannot prove their oncogenicity be excluded from these new journals?

Irrespective of the semantics, research on oncogenes remains fashionable and important, and workers in the field have met increasing difficulties in identifying the most appropriate and accommodating outlets for their results. The launch of *Oncogene* and *Oncogene Research* was therefore widely welcomed. But having two publications, and with such confusingly similar names, immediately creates a dilemma. Which will be regarded as the more prestigious? More seriously, can they both survive?

The editorial aspirations are virtually identical, both journals promising high-quality, peer-reviewed papers, spiced with topical reviews (of which there have been rather few) and with relatively rapid publication times. In the volumes provided for appraisal, both can claim to have delivered, the standards being roughly on a par with *Molecular and Cellular Biology* or the *EMBO Journal*, but judging from their thickness, neither appears to have been inundated with contributions. There is little to choose in the quality of papers that have appeared thus far, and nothing to choose in the calibre of the two international editorial boards; indeed some names appear on both boards.

At the level of principal editors, *Oncogene Research* might claim the edge, both in number and in international standing, but in marketing terms *Oncogene* wins hands down. Copies of *Oncogene Research* have been sporadic to say the least, while its competitor now boasts a regular monthly issue. The A4 format of *Oncogene* (maybe just a little too big), the high quality

Oncogene RESEARCH

reproduction and a style that emulates both *Cell* and *EMBO Journal* make it very attractive, while, to my mind, the undoubted quality of *Oncogene Research* is offset by its rather old-fashioned air. I gather there are plans for a relaunch in new livery, but unless this is accompanied by a more reliable publication schedule, I see the balance tipping in favour of *Oncogene*.

If there is to be a circulation war between these two fledgling journals, then it will eventually be won or lost on the quality of papers each can attract. Locked in combat, they may be forced to compromise their standards while an uneasy truce would leave two slightly lame survivors. As neither will be the outlet of choice for the headline stories, and there may not be enough good copy to sustain them both, the scientific constituency these journals represent would be best served by a merger. □

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Divided advantages

Michael R. Hanley

Molecular Brain Research. Editor-in-chief D.P. Purpura. Elsevier. 4/yr. Dfl 299, \$145.75.

Molecular Neurobiology. Editors Nicholas G. Bazan and David C. U'Prichard. Humana. 4/yr. \$100.

THE term 'molecular neurobiology' first became respectable following the 1983 Cold Spring Harbor symposium of the same name. Before that, anyone who put the two words together was treated as being a little weird and clearly out of touch with reality. But times have changed, and neurobiology, like much of biology as a whole, has 'gone molecular'. The boundaries of this hybrid subject are not well defined, but generally it subsumes much the same material to be found in the table of contents of Cold Spring Harbor's book-of-the-meeting, technical innovations in molecular genetics, cell culture, developmental biology and electrophysiology included.

Molecular Brain Research is another addition to Elsevier's *Brain Research* stable. The idea of splitting *Brain Research* into smaller operations, although motivated by the expansion of neuroscience, clearly has the benefit of bringing the price down to a terrestrial level. The voluminous parent journal is incredibly expensive — whether or not the publishers realize it, they are performing the experiment of finding out just when their journal will be priced out of viability. *Molecular Brain Research* is altogether better value.

Biologists like the notion of finding a speciality to call their own, and most journals have to do so if they are to survive. Sometimes it happens spontaneously, as publications with a particular title inexplicably become the favoured setting for a subset of manuscripts that might suggest another title. Thus, *Molecular Brain Research* is in fact the *Journal of Neural RNA*. Much of the early content has focused on Northern blots, *in situ* hybridization and expression of mRNA in oocytes. Although not deliberate, this is clearly emerging as the journal's special province, and, on the whole, the published material is of a high scientific standard. Another hallmark of the parent journal is the long lag times between submission and appearance of contributions. A quick survey of the papers in *Molecular Brain Research* suggests that the editors are doing rather better here, with delays of 4–7 months between submission and publication.

Molecular Brain Research is unlikely to appeal to those outside the neuroscience community. But it is a modest and

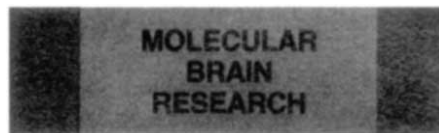
sensible addition to an already successful group of journals.

Molecular Neurobiology, a review journal, is a different proposition. I was only too well aware of its impending arrival, having been inundated with announcements addressed to Dr Hanley, Hanley, Hanley and every other permutation of my name. This is a publication which has the stated goal of becoming "the leading intensive review journal in the field" — which should not be hard to achieve as it is the only such journal in the field.

First impressions are unfavourable. The cover carries not the usual natty photo or simple graphics job with soothing colours, nor even a run-down of authors and contents. Rather we have a stark list of members of the editorial board printed on a lurid red background, suggesting that this is what the journal is really about — putting one's name before the public. As there are twice as many editors as papers appearing in a given year, it seems more appropriate to see the list on the cover as a list of those who get the journal free.

The cover could be forgiven as a sign of insecurity, but second impressions are no better. The inaugural editorial states that the journal will encompass, "at the molecular level" (of course), "physiological processes", "integrated functions" and "behaviors". This sounds a lot like plain old everyday neurobiology, and encapsulates the main difficulty this journal will

experience — how to get enough material for review and yet be 'molecular'. The contributions themselves are predictable in content and are of varying quality. The opening review, "Molecular Neurobiology: Past, Present, and Future", is very poor, and looks as if it travelled from author, to typesetter to printer with no editorial intervention. That article



marked a nadir, but although later papers are better in style and content most of them cover familiar ground.

The application of new technologies to the traditionally boggling issues of neuroscience has become a high-profile business, and most good papers are snapped up by prominent journals with a wide circulation. There is enough decent science left over to sustain *Molecular Brain Research* in addition to the broad range of outlets for work in neurobiology and neurochemistry. But, to my mind, there is no need for a publication devoted solely to reviews of the molecular biology of the nervous system, given that there are already plenty of places for such pieces to be published. □

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Aid on AIDS

Fred S. Rosen

AIDS. Editors M. W. Adler, J. Gold and J. N. Weber. Gower Academic. 6/yr. £100, \$170 (institutional); £60, \$90 (personal).

OF RESEARCH on any subject, it is that on the various aspects of AIDS that one would expect to be growing most rapidly. Here, more than in any other area, the launch of a journal is justified, especially when it is of as wide appeal as this one.

The editors' intention is to cover the subject broadly — epidemiology, geographical medicine, immunobiology, virology, clinical medicine and pharmacology — and original papers on these topics have been published from authors in North America, Europe and Africa. There have also been several short but authoritative review articles on such subjects as paediatric AIDS and Kaposi's sarcoma. So far, the scientific standard of the contributions has been high.

The journal has other features which will enhance its chances of doing well over the long term. Each issue contains a bibliography of the current world literature,

over 250 journals being scanned for articles on AIDS. In addition, statistics from the World Health Organization and the Centers for Disease Control are published regularly — readers may be dismayed to learn that Albania is the only AIDS-free spot in Europe; for those seeking more agreeable climes, Montserrat and the Seychelles also are reported to have no AIDS.

Even given that many papers on AIDS and associated topics are creamed off by the more general, fast-moving science journals, it is surprising that more publications have not been started to deal specifically with the subject. *AIDS* does so, and does so well. The editorial board is international in composition, and among its members are prominent figures covering all of the main areas of interest; as long as they stay involved, the journal should continue to provide global coverage of the scientific investigation of AIDS. Scientists, clinicians and public health workers should all consider submitting their papers and subscribing to it. □

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Need for speed of transmission

David A. Brown

Synapse. Editor-in-chief John E. Johnson. Alan R. Liss. 6/yr. US \$240, elsewhere \$272.

Neuroscience Research Communications. Editor W. H. Gispen. Wiley. 6/yr. £85, \$150.

How many journals of neuroscience do we need? Well, there are about 11,600 members of the Society for Neuroscience. Suppose each writes (on average) one paper per year. Add on the fruits of the labours of those in Europe and Japan who are not members of the society, and we're talking in terms of around 20,000 papers a year, a number that is still increasing. Hence, ever more journals.

These two newcomers have two features in common: both cover 'general' neuroscience, and both are published bimonthly. In other respects they are totally different.

Synapse is a 'serious' journal: it has a prestigious (though almost exclusively North American) editorial board, and

publishes full-length papers in proper typeset form. Its aims are to carry "articles dealing with all aspects of synaptic structures and function [including] neurotransmitters, neuropeptides, neuromodulators, receptors, gap junctions, metabolism, plasticity, circuitry, ion chemicals, patch recording, development, pathology, toxicology, etc" — a pretty comprehensive list, in which the "etc" seems a bit superfluous. Its principal competitors are therefore such publications as the *Journal of Neuroscience*, *Neuroscience* and *Brain Research*. In fact, it most closely resembles *Journal of Neuroscience* in format (double-column pages on glossy paper, but with slightly smaller print), and in content. There is, perhaps, a shade more emphasis on the neuroanatomical — not surprisingly, given the main interest of the editor-in-chief (J. E. Johnson of Johns Hopkins) — but less so than in *Neuroscience*.

I have a couple of niggles: review articles are accepted but these are not always separated geographically from research articles and are not clearly marked as such in the index; and the appearance of the journal would be improved if the publisher's staff labelled the figures, instead of leaving it to the whims of the authors.

Connecting up

Howard L. Weiner

Brain, Behavior, and Immunity. Editor-in-chief Robert Ader. Academic. 4/yr. \$120, £83.

THE term 'psychoneuroimmunology' refers to the interfaces of the nervous, immune and endocrine systems as they are affected by behaviour and the environment. Although this is a somewhat difficult field to define, it appears that it has come of age.

The scientific basis of psychoneuroimmunology rests on the facts that there are receptors for neurotransmitters and hormones on lymphocytes, the nervous system and the endocrine system; that changes in nervous system electrical activity have been associated with immunological events; and that organs of the immune system are innervated by the nervous system. Given this wealth of connections, a variety of areas of investigation are possible. Furthermore, with increasingly sophisticated tools becoming available, an attempt can now be made to dissect the systems involved and isolate the factors which affect their functioning.

Brain, Behavior, and Immunity is designed to provide a forum for scientists working on these questions, and perusal of the four issues of Vol. 1 suggests that the journal is off to a good start. In general

the quality of the contributions is high, and they have come from scientists in a variety of disciplines, immunology, neurobiology, pharmacology and psychiatry among them. Some of the investigations are basically orientated, for example those dealing with somatostatin receptors on lymphocyte populations and innervation of the thymus gland. Others are attempts to study the interaction between



different systems, such as the effects of hippocampal lesions and neuropeptides on immune responses. Others again are reports of such phenomena as impaired natural killer cell activity during bereavement and behaviourally conditioned enhancement of delayed-type hypersensitivity in the mouse.

The journal's standard of production is high, and each issue contains a stimulating editorial or comment. For those interested in psychoneuroimmunology, *Brain, Behavior, and Immunity* is an excellent medium through which to follow progress in this newly emerging discipline. □

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These points apart, I found the format attractive, and the papers I read were of generally good quality, no doubt in part because some members of the editorial board are doing their inaugural duty in publishing in it. The contents are indexed in *Current Contents* and *Index Medicus*, so potential authors know that their work may well reach its intended public.

Will *Synapse* succeed? At the moment, it is in the second division — not because

SYNAPSE

John E. Johnson, Jr., Editor-in-Chief

of the quality or variety of the papers, but because, with a bimonthly publication schedule and only ten of so contributions per issue, it just does not contain enough material to put it on the compulsory purchase list for hard-pressed libraries. Promotion to the first division of required reading must therefore depend on more papers and more frequent publication.

Neuroscience Research Communications is a different kettle of fish altogether. This is another publication of the 'quickie' variety, where authors have to produce oven-ready copy, either typed or on disk if they use *Wordstar* or *WordPerfect* (why so limited a software choice — haven't Wiley got other, more scientific, software?). It is printed on glossy paper, so photographs reproduce well.

The main competitor is probably *Neuroscience Letters*, which costs much more but appears fortnightly, is properly typeset and publishes quickly. Moreover, although it is uneven in content, it frequently contains some very good papers (probably because it's one of the most convenient places to send rejects from the prominent general-science weeklies). I cannot envisage *Neuroscience Research Communications* achieving that sort of status for a long time yet. My suspicion is that there is a place for a neuroscience equivalent to *Biochemical and Biophysical Research Communications*, but if this journal is to fill the slot it will have to move to a monthly schedule, and quickly. □

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Journal prices

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1989. This information is not complete in all cases; an individual rate may be available, for example, or there may be an additional charge for air-mail delivery. Readers interested in a particular journal should therefore check prices with the publisher before subscribing.

On life and death

Mary Midgley

Bioethics. Editors Helga Kuhse and Peter Singer. Basil Blackwell. 4/yr. UK £44, North America \$93.50, elsewhere £54.50 (institutional); UK £21.50, North America \$44.50, elsewhere £25.75 (personal).

THIS international and interdisciplinary journal was started in January 1987 to deal with "the ethical aspects of issues raised by medicine and biology". Such a publication is badly needed to cope with the various headaches raised by the recent explosion of biological and medical technology — problems for which the word 'bioethics' has itself lately been coined. As the editors reasonably remark, we can no longer rely on "the general trust in medical practitioners and scientists" because these specialists themselves often see grave difficulties in the issues with which they grapple, and they cannot take the responsibility of decision alone. The rest of us will need to do some new thinking, with all the painful effort that thinking involves.

What does *Bioethics* cover? The editors exclude ecological problems, confining their subject matter to human life and death. They concentrate especially on



euthanasia, along with new techniques of prolonging life; new reproductive techniques — *in vitro* fertilization, sex selection, genetic engineering and so on; ethical constraints on research, whether with patients, human embryos or animals; patient autonomy and consent to treatment; and allocation of scarce medical resources, both at individual and at national level.

This seems a good spread. There is, however, an imbalance in the journal's actual coverage because contributions on the first two topics — the beginning and the end of life — outweigh those on the others. There is, I think, some legacy here from the days when *a priori* debates about abortion and euthanasia were hotly carried on by theologians and lawyers, in abstraction both from all other topics and from the complex web of awkward facts involved. In general, though, the articles and book reviews in *Bioethics* represent a vigorous move away from these polemics to a far more realistic and fruitful approach. They are clear, forceful and lively. Some of them simply report current changes — for instance, the progress of euthanasia in the Netherlands, and the growth of a large commercial market in

organs for transplant in the Third World. Some draw attention to neglected matters, such as the strange effects of infertility treatment on the lives of the women involved. Others discuss more theoretical issues, such as the extent to which various practices, from AIDS testing to the prescription of psychotropic drugs, threaten civil liberties. But all make a real and useful attempt to balance the theoretical and the practical aspects.

How important is this journal for non-specialists? We are all potential patients; and even when we manage to avoid that fate, the gradual spread of medicine into more and more areas of life keeps posing new ethical questions. The difficulty is to

make the debates co-operative and constructive rather than simply clashes of propaganda, and *Bioethics* seems to me to make an uncommonly good shot at this difficult goal. The editors try hard for balance. The fact that their contributors sometimes show either a crusading passion for progress or a crusading certainty that the boat is going the wrong way simply reflects the condition of our age. The discussions in *Bioethics* can certainly help us to understand that condition. □

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Conforming to type

Robert B. Freedman

Protein Engineering. Executive editors A.R. Rees and G.A. Petsko. IRL. 8/yr. UK £100, North America \$190 (institutional); UK £45, North America \$85 (personal).

SOCIOLOGISTS of science point to the founding of specialist journals and the institution of regular specialist international meetings as indicators of the emergence of new areas of research. If that is so, then IRL Press is betting heavily that protein engineering will emerge as a dominant field in the 1990s and beyond.

In early 1987, IRL launched both a journal and meeting series on the subject, and the journal is now well into its second volume. The masthead of Vol. 2 No. 2 shows an expansion of the central editorial team to two executive editors, three associate editors and one "Commentary" editor, with representation from the United States (three), and Britain, Japan and the Soviet Union (one each). The editorial board is broadly based and representative of activity in the field, but the journal has had teething troubles. Editorial comment in the last issue of Vol. 1 makes it clear that the flow of good-quality papers has not been as rapid as was hoped, and the editors' insistence on maintaining high standards has resulted in thinner issues.

The journal's scope is wide. A large number of papers are theoretical analyses of protein sequence and structure aimed at structure prediction or the improvement of methods for structure prediction. Others deal with protein structure determination, both by X-ray diffraction and nuclear magnetic resonance, or physico-chemical studies on protein folding and stability. But the strongest theme is that most widely associated with protein engineering, namely the exploration of

protein structure and function through analysis of the properties of modified proteins. Many of the contributions on this topic deal with mutant proteins derived by site-directed mutagenesis, but other methods of 'engineering', such as total gene synthesis and protein semi-synthesis, are also well represented. Overall, the coverage is interesting, the journal is attractively produced, and each of the eight issues published to date has included several papers which I have found stimulating enough to look at closely.

So, *Protein Engineering* is good, but is it necessary? Most of the papers that have appeared to date would have been perfectly at home in *Journal of Molecular Biology* or in *Biochemistry*, and certainly all would have been welcomed in the rival newcomer *Proteins*. But this does not mean that *Protein Engineering* is superfluous. The editors are making a clear effort to define and appeal to a specific constituency with certain interests in common. This emerges most clearly in the Commentary contributions. Some of these have been meeting reports and others have been discussions of particular papers, along the lines of the articles in *Nature's* News and Views section. But others again have been small focused reviews, or discursive pieces on topics such as nomenclature; there has even been one full-scale, vigorous debate between Estell of Genentech and Fersht of Imperial College on the use of linear free-energy relationships in analysis of mutant enzyme kinetics.

The Commentaries were given a good start by their editor, Ron Wetzel, and have been consistently readable and pointed. Their concerns and approach will be shared by many people now developing interests in protein engineering, and if their tone and the quality of the original papers can be maintained, then *Protein Engineering* will grow and prosper with the field itself. □

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Living images in print

David Gadian & Graeme Bydder

Reviews of Magnetic Resonance in Medicine. Editor-in-chief John C. Gore. Pergamon. 2/yr. £60, \$100 (institutional); individual rates also available.

Journal of Medical Imaging. Editor-in-chief H.E. Schütte. Elsevier. 6/yr. Dfl 325, \$158.50.

It is only three years since two new journals dealing with aspects of magnetic resonance in medicine were reviewed here (*Nature* 317, 299; 1985). Now we have two others in a similar or related area. Are there too many? Possibly, but this is still a rapidly growing field.

Ultimately, the success of any journal is determined by the quality of its contents. This is particularly true for such a publication as *Reviews of Magnetic Resonance in Medicine*, for invited reviews may be uneven in quality and usefulness, and may appeal to very different audiences. The first three issues of this journal contain

JOURNAL OF MEDICAL IMAGING

just seven articles, taking about 350 pages, and although they do show some variability some excellent work has been published. Thus although the very first review (by K. Wuthrich), on the structure and function of proteins and nucleic acids, may appear to be on the fringe of applications to medicine, it was appropriate to begin with a review of this subject, especially because NMR techniques that are initially developed for structural studies may subsequently prove valuable in imaging or metabolic studies. Particularly noteworthy articles, more directly related to *in vivo* studies, have been on localization methods for *in vivo* NMR spectroscopy (W.P. Aue) and on artefacts in magnetic resonance imaging (R.M. Henkelman and M.J. Bronskill).

We understand that publication is somewhat behind schedule, but the contributions remain up to date. For example, that by Henkelman and Bronskill (first issue of 1987) contains many references to work published in 1987. *Reviews* covers all aspects of magnetic resonance in medicine, and although papers are generally invited, there is also scope for authors to submit contributions. Here is a journal that shows considerable promise.

Journal of Medical Imaging covers clinical aspects of radiology and medical imaging in the broadest sense, including

Enzymes that work

Christopher R. Lowe

Biocatalysis. Managing editor David Best. Harwood. 4/volume. UK £164, North America \$268, elsewhere £204 (corporate); UK £110, North America \$182, elsewhere £138 (library); UK £56, North America \$90, elsewhere £70 (personal).

THE information explosion in biotechnology threatens to overwhelm even the most ardent of readers among us. Any new journal, therefore, stands a good chance of being criticized as simply adding to the myriad of pseudo-biotechnology publications already in the market place and fuelling the competition for that scarcest of all resources, the quality manuscript.

The managing editor of *Biocatalysis* is clearly sensitive to these issues and has, quite sensibly, focused his journal specifically on the underlying science and potential industrial exploitation of biological catalysts for the interconversion of chemical species. The advent of the journal is timely, because of the intense worldwide interest in biotransformations directed towards the production of new synthetic routes.

The topics covered include the region and stereospecificity of biological catalysts, the reaction conditions under which they operate, the development of biocatalytic systems from discovery to commercialization, the integration of chemical and biological processes to produce novel synthetic routes, and the engineering aspects of the industrial implementation of these systems.

Inevitably, the scope of *Biocatalysis* overlaps somewhat with that of both established and newly introduced journals,

notably *Enzyme and Microbial Technology*, *Journal of Chemical Technology and Biotechnology* and *Applied Biochemistry and Biotechnology*, but there is much to be said for having a choice of journals in an actively expanding area of interdisciplinary research. The declared intention of *Biocatalysis*, to act as a focal point for chemists, biochemists, microbiologists, chemical engineers and biochemical engineers, is soundly based.

The journal has an unmistakable international flavour, with a well-balanced selection of regional editors and members of the editorial board. Each issue has contained five to nine articles, some of them short reviews, some primary research papers. Not unexpectedly, many of the contributions published to date have originated from members of the editorial board, but these are distinguished authors and their papers have been of a consistently high quality. A welcome feature is the grouping of the solicited reviews into 'themes', the first series being collectively entitled "Biocatalysis in Multiphase Environments". The format of the journal is easy and readable, and (much to my personal delight) the titles of work cited are included in the references.

In all, *Biocatalysis* should appeal to a substantial body of readers involved in applied enzymology. Those self-same readers, however, might well also be interested in other aspects of the 'applied' literature, most notably that dealing with patents, and the editors might consider including some review of this literature, perhaps to appear in the thematic issues. But this mild criticism should not deter readers from subscribing to what may become a very successful journal. □

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REVIEWS OF MAGNETIC RESONANCE IN MEDICINE

nuclear medicine, ultrasound and magnetic resonance imaging. The journal contains review articles, descriptions of new techniques, research papers and case reports. The delay between submission and publication in Vol. 2 is typically 5–12 months, allowing for revision in most cases. Although this is the official publication of the Netherlands Society of Radiodiagnosis, it has attracted contributions from Hong Kong, Japan, the United States and many European countries other than the Netherlands. Interestingly, in the advertisements Toshiba offers products conducive to the Dutch 'budget unfriendly' climate.

There are now over 60 radiology

journals, and *Journal of Medical Imaging* is unusual among the newer ones in that it covers the entire subject — with the exception of the *European Journal of Radiology*, journals that have appeared over the past ten years have specialized in topics such as ultrasound, computed tomography or magnetic resonance imaging. So the newcomer will compete with much larger and better established publications. It will probably achieve a respected position among European general radiological journals alongside the likes of *Clinical Radiology*, to which it is most similar in scope and aims. □

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Developed tastes

Donald D. Brown

Development. Editor-in-chief Chris Wylie. *The Company of Biologists*. 12/yr + supplements. UK £355, North America \$780, elsewhere £405 (institutional); UK £90, North America \$145 (personal).

Genes & Development. Editors G. Bulfield and M. Mathews. *Cold Spring Harbor Laboratory*. 12/yr. \$220 (institutional); \$90 (personal).

THE state of any field is reflected in the quantity and quality of the publications that serve it. The recent appearance of two excellent new journals emphasizing developmental biology is further confirmation of its growth and maturity.

Developmental biology is not a scientific discipline, but a set of questions that has been asked since the beginning of this century and is now yielding to the methods of biochemistry, genetics, cell biology, immunology and molecular biology. So journals dealing with embryos no longer appeal just to a few specialists, but to researchers in many areas of modern biology.

Those who want to keep up with advances in developmental biology must not only be versed in its contributory disciplines, but also in the life cycles and special

advantages of a multitude of organisms (plants and animals). The reason for this is that there is no 'phage for development', that is, no single organism in which we can answer all of the questions we wish to ask. There are so many important biological systems that are limited to certain organisms or that have been well studied in a particular organism and are the subject of a wealth of descriptive data accumulated over the years. The new methods permit a fresh attack on previously intractable problems in the organisms of choice.

Considering these ground rules, there is plenty of room for two new journals in this broad and expanding field. *Development* is the continuation of *Journal of Experimental Embryology and Morphology* (or JEEM as it was called). Experimental embryology was the dominant subculture of the field in the first half of the century. It was as near as the field came to being a discipline in the sense that experimental embryology spawned its own set of techniques and methods, and emphasized its own special organisms — chickens, frogs and sea urchins. Experimental embryology is alive and well today but is dependent upon the helping hand of the new biological methods derived from other disciplines. The journal has broadened its scope and the editors welcome papers on all organisms and on subjects ranging from the morphological to the molecular aspects of development. The quality of the contributions since the journal changed its title in 1987 has been very high, and the papers have been of general interest.

Genes & Development is the first journal to be published and administered by Cold Spring Harbor Laboratory. It, too, is broadly based in subject matter but as its title suggests it emphasizes contributions in molecular genetics that elucidate the structure and control of genes in development. We have come to expect high quality and timeliness from Cold Spring Harbor's many symposium volumes, and the journal maintains that standard.

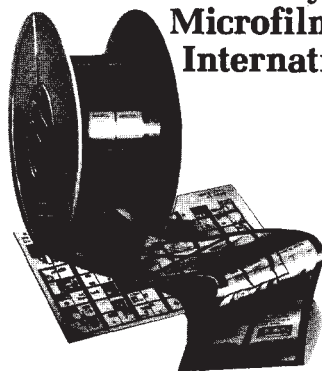
Both journals publish reviews, meeting reports, book reviews and commentaries along with the usual research articles (to date, the reviews in *Development* have been especially distinguished). Both pay considerable attention to the quality of their pictures, and both put a stress on rapid publication, which these days seems essential if a journal is to compete for the best manuscripts. Neither *Development* nor *Genes & Development* can be said to fill a void; I can count ten other journals with overlapping subject matter, but there is no lack of high-quality papers to fill them. In less than two years, these two newcomers have become essential reading for modern research biologists. □

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Among other titles submitted

Advanced Robotics (VSP); *American Journal of Noninvasive Cardiology* (Karger); *Aphasiology* (Taylor & Francis); *Applied Cognitive Psychology* (Wiley); *Argumentation: An International Journal of Reasoning* (Kluwer); *Arid Soil Research and Rehabilitation* (Taylor & Francis); *Blood Reviews* (Churchill Livingstone); *Cardiovascular Drugs and Therapy* (Kluwer); *Clinical Vision Sciences* (Pergamon); *Comments on Agricultural and Food Chemistry* (Gordon & Breach); *Computational Mechanics* (Springer); *Computer Speech & Language* (Academic); *Distributed Computing* (Springer); *Environmental Software* (Computational Mechanics Publications); *Epilepsy Research* (Elsevier); *European Journal of Personality* (Wiley); *Fetal Therapy* (Karger); *Food Hydrocolloids* (IRL); *Future Computing Systems* (Maruzen/Oxford University Press); *Hematologic Pathology* (Marcel Dekker); *Hematology Reviews and Communications* (Harwood); *Hydrological Processes* (Wiley); *International Journal of Computer Vision* (Kluwer); *The International Journal of Supercomputer Applications* (MIT Press); *Irrigation and Drainage Systems* (Kluwer); *Journal of Adhesion Science and Technology* (VSP); *Journal of Clinical Laboratory Analysis* (Alan R. Liss); *Journal of Clinical Research and Drug Development* (Elsevier); *Journal of Electromagnetic Waves and Applications* (VSP); *Journal of Fluids and Structures* (Academic); *Journal of Psychopharmacology* (Oxford University Press); *Journal of Psychophysiology* (Oxford University Press); *The Journal of Supercomputing* (Kluwer); *Journal of Voice* (Raven); *Lanthanide and Actinide Research* (Kluwer); *Lasers in the Life Sciences* (Harwood); *Mathematical Engineering in Industry* (VSP); *Mechanical Systems and Signal Processing* (Academic); *Medical and Veterinary Entomology* (Blackwell Scientific); *Metabolic Brain Disease* (Plenum); *Molecular and Cellular Probes* (Academic); *Natural Product Updates* (Royal Society of Chemistry); *Nuclear Geophysics* (Pergamon); *The Pain Clinic* (VSP); *Pediatric Reviews and Communications* (Harwood); *Probability in the Engineering and Informational Sciences* (Cambridge University Press); *Professional School Psychology* (Lawrence Erlbaum); *Psychology & Health* (Harwood); *Regulated Rivers: Research & Management* (Wiley); *Science in Context* (Cambridge University Press); *Serodiagnosis and Immunotherapy* (Academic); *Soviet Agricultural Biology Part 1 Plant Biology, Part 2 Animal Biology* (Allerton); *Surgical Research Communications* (Harwood); *Water Quality International* (IAWPRC); *Water Resources Management* (Kluwer).

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